

Strategy for retinal cell therapy

Patient-centered risk assessment

Masayo Takahashi
Vision Care group. Kobe City Eye Hospital



The first-in-man application of iPS-derived cells
to treat wet AMD (2014)

Autologous iPSC-RPE transplantation



Nature's 10
Ten people who mattered
this year. 2014





JAPAN'S BIG BET ON STEM-CELL THERAPIES MIGHT SOON PAY OFF

Induced pluripotent stem cells are being tested to treat blindness, paralysis, Parkinson's disease and more. Approvals might be around the corner. **By Smriti Mallapaty**

Japan is brimming with signs of an approaching medical revolution. Shiny white robots are tending dishes of cells, rows of incubators hum in new facilities, and a deluxe, plush-carpeted hospital is getting ready to welcome its first patients.

Building on the Nobel-prizewinning work of stem-cell scientist Shinya Yamanaka, researchers across the country are crafting cells into strips of retina, sheets of cardiac muscle or blobs of neurons, in the hope of treating blindness, mending hearts and reversing neurodegeneration. Results from early-stage clinical trials — some announced just in the past few weeks — suggest that the cells might actually be working to treat conditions as varied as Parkinson's disease and spinal-cord injury.

Now, after nearly two decades of hard work and setbacks, many say that Japan is on the cusp of bringing these therapies to market.

Yamanaka, who runs a lab at Kyoto University, discovered in 2006 that adult cells could be reprogrammed into an embryonic-like state,

capable of becoming practically any kind of tissue. These induced pluripotent stem cells — or iPSCs — won Yamanaka the Nobel Prize in Physiology or Medicine in 2012, and propelled him to superstar status. They have become a symbol of the country's global scientific aspirations.

The Japanese government has poured more than ¥100 billion (US\$560 million) today into research and development on regenerative medicine, one of billions more from private funders, organizations and companies. "People thought, 'Now we can create any incurable disease,'" says Shinya Yamanaka, director of Kyoto Health University Hanada Clinic. "There was so much hype."

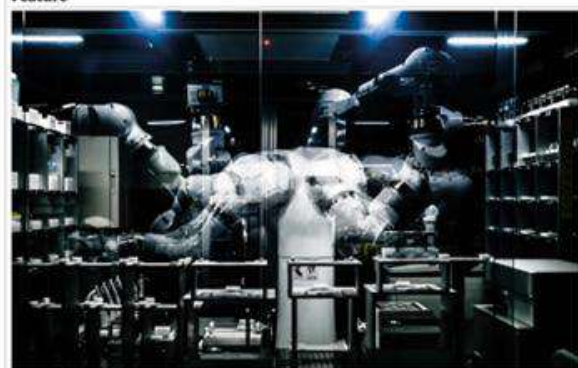
Scientists launched clinical trials and start-up firms. Large biotech companies swooped in, investing even more in manufacturing hubs. Now, medical facilities are preparing to welcome a rush of patients from Japan and abroad. "Regenerative medicine in Japan is moving very dramatically," says Masayo Takahashi, an ophthalmologist at Keio City Eye Hospital and president of the biotechnology company, Vision Care. In 2024, she became the first to treat someone with cells derived from iPSCs.

There are more than 60 iPSC-cell clinical tri-



Masayo Takahashi ran the first clinical trials for iPSC cells. Three individuals who received the treatment, the cells have survived and are only one year after surgery. But the signs of benefit are mixed. One of the three individuals said she could see her husband's face clearly for the first time in ten years, but only through a wall.

Feature



This robot in Keio is preparing cells for transplant to treat people with muscular degeneration.

als underway. The researchers presented preliminary results — not yet peer reviewed — at a press conference in March, showing that one individual with paralysis can now stand independently and is learning to walk. Another can move some of their arm and leg muscles but cannot stand. Two others did not show substantial improvements.

Similar trials are under way outside Japan, some of which involve many more participants than the Japanese trials. But unlike other regions, Japan has made the path to approval relatively easy, says Clive Svendsen, a stem-cell researcher at Cedars-Sinai Medical Center in Los Angeles, California. In 2015, Japan introduced a regulatory path for regenerative biotech products called conditionally approved if they are shown to have no major safety issues and to be likely to be effective.

Companies can offer the treatments, with costs mostly covered by the national health system. But they must continue to collect data on safety and efficacy to remain "conditionally approved."

Some researchers have raised concerns about this fast-track process and related programs in Japan. Last year, two of the four products that had received conditional approval under this mechanism — one involving high-magnesium for tremors in the hand, the other agones therapy to treat diabetes in approved areas in the limbs — were withdrawn.

The first was rejected for formal approval after nearly a decade on the market because it lacked to show clinical benefit. The second was withdrawn about five years after being conditionally approved, because surveillance data did not reproduce results observed in earlier trials.

Hirotaka Kawaguchi, an orthopedic surgeon at Nishigaki Hospital in Kadoma, says he is concerned that the fast-track process shifts the burden from pharmaceutical companies, which would otherwise have to conduct large-scale trials, to the public treasury, which therapy for expensive, unproven treatments. Last year, Japan's Ministry of Health, Labour and Welfare issued guidance documents that clarified that conditional approval should not be the ultimate goal for companies.

Others are less concerned about Japan's fast-track process for conditions that are rare or have few other treatment options. "In order to meet this [goal] as quickly as possible, you're going to have to have an element of risk," says Svendsen. "What I see in Japan has been pretty amiable; they are putting regulations in place."

iPSCs cells for all

Even without approvals in hand, the industry is building capacity in the expectation that demand for these treatments will be high by 2026. Sumitomo Pharma completed construction of what it describes as the world's first manufacturing facility for donor-derived iPSC-cell products. The building in Osaka, which looks like a glass floating shelter, is 2020, 6 kilometers from Keio City Eye Hospital's site. The company is also supporting two early-stage Parkinson's trials in the United States.

Masayo Takahashi has chosen a more portable manufacturing model for her muscular degeneration treatments: a white, muscular-looking, two-wheeled robot. Powered by machine learning, it checks in on cells' progress as they are prepared for transplant through a microscope. In 4 months, it can produce enough cells for more than 900 individual treatments.

Developed together with Keio's Takahashi (no relation to Jun or Masayo), a computational biologist at the RIKEN Center for Biogenetic Dynamics Research in Kobe, the robot ensures that cell-culture techniques developed in a laboratory can be reproduced anywhere, thus avoiding the logistical uncertainties that delivering the cells would entail. "It is easy to transfer our treatment to the world," says Masayo Takahashi, who hopes to partner with groups across Japan and Asia.

But for many, the ultimate goal of iPSC-cell therapies is to remain to the identified version of autologous transplants. Such transplants would reduce the risk of rejection and avoid the need for immune-suppressing drugs over extended periods. They could also address some ethical worries that have been raised, such as the idea that transplanting another person's tissues into the brain is changing someone's identity, says Jun Takahashi. "Our ultimate goal is autologous transplantation," he says.

Of more than 600 individuals who have received iPSC-cell-derived products in trials globally, only 11 have had autologous transplants, according to an analysis by Melissa Carpenter, president of the stem-cell-focused Carpenter Consulting in Seattle, Washington. It's possible that there have been more recipients than this, because many firms have not disclosed figures, says Carpenter.

But often, companies do not prioritize the autologous route, because production of these treatments is just too costly. One project by the CIRA Foundation is trying to change that. Tucked away in a high-rise building in Osaka, the mSP3 project aims to create autologous iPSCs at a cost of \$1 million per patient, much cheaper than comparable therapies.

On one floor of the new facility — set up in 2024 — four cell-culturing machines sit behind glass walls. Each can transform adult cells from a patient into enough iPSCs cells for a personalized treatment in about a month.

The room is designed to hold 48 machines, with space for another 150 next door. Masayo Takahashi, who heads the project, is confident that the foundation will meet the

Nature 2025.4.17 "JAPAN's big bet on stem-cell therapies might soon pay off"

6 million target for creating autologous cells by the June, but differentiating the cells into a desired cell type costs many millions more. The goal is to show that combined figure drops to \$1 million.

Safety first

So far, stem-cell therapies have proved safe, say researchers. Although there have been concerns about the process of creating iPSCs could make them prone to forming cancers, safety guards have been established to ensure the process to ensure that no pluripotent cells remain in a transplant, and that cells for transplant have been screened for cancer-causing gene mutations, says Shimamura.

"Remarkably, of all the patients that have been implanted, there were no serious events that were related to the product that we could find," says Carpenter. "Autologous results from clinical trials approved by regulatory authorities globally. Still, the fear persists. And any adverse mutation in the cells are double- and triple-checked."

One recent scare involved a trial, led by Shimamura, in which a 73-year-old man received

an implant to replace cells that line the inner surface of the cornea, derived from donor iPSCs, to treat a form of swelling of the eye. The treatment proved safe, and the individual's vision became low blurry, but Shimamura had to end the study abruptly.

The iPSCs came from the CIRA Foundation and were created at the time when no mutations known to cause cancer. Genetic sequencing after the cells had been cultured into corneal endothelial cells was also done, but sequencing just before the transplant revealed a deletion in the TP53 gene, which is considered a "oncogene change." Investigations by Shimamura's team revealed an adverse event related to the mutation, and he said he saw how likely cells containing the mutation weren't more likely to be dangerous.

"It's possible that regulatory agencies will now require all trials to undergo whole-genome sequencing of the cells just before transplantation. "It's good to be safe," says Shimamura. But, he adds, researchers and regulators need to figure out how they're going to cope with these minor mutations."

OUR ULTIMATE GOAL IS AUTOLOGOUS TRANSPLANTATION.



Jun Takahashi is trying to treat Parkinson's.

Medical travel

The stakes are high, as are the costs. Masayo Takahashi's company plans to seek regulatory approval for its muscular-degeneration treatment following Japan's trial. But before that, she plans to collaborate with physicians to start therapies through government programs that would require that individuals, and not the national health system, pay for most of the therapy. The price tag for such therapies, which some have paginated \$1 million, makes likely that this would be an option only for wealthy Japanese people and medical tourists. Takahashi is also looking at whether the procedure could be covered by private health insurance.

The first site that could start offering these advanced therapies is Fujita Health University Hanada Clinic, where Shimamura worked. A nearby facility, a joint venture between Fujita's Hanada International Airport.

Yuko Ozawa, an ophthalmologist at the clinic, is collaborating with Takahashi and identifying potential patients. She is confident that the right ones will pay off. "People might hesitate to accept the treatment at first, she says. "But after several successful cases, they will come."

Smriti Mallapaty writes for Nature from Brisbane, Australia.

1. Takahashi, J. & Yamanaka, S. Cell 181, 610–620 (2024). doi:10.1016/j.cell.2024.03.016
2. Yamanaka, S. Cell 147, 281–283 (2012). doi:10.1016/j.cell.2012.02.037
3. Yamanaka, S. Cell 147, 281–283 (2012). doi:10.1016/j.cell.2012.02.037
4. Yamanaka, S. et al. Nature 460, 475–479 (2009). doi:10.1038/460475a
5. Yamanaka, S. et al. Nature 460, 475–479 (2009). doi:10.1038/460475a
6. Yamanaka, S. et al. Cell 147, 281–283 (2012). doi:10.1016/j.cell.2012.02.037

Gartner's hype cycle

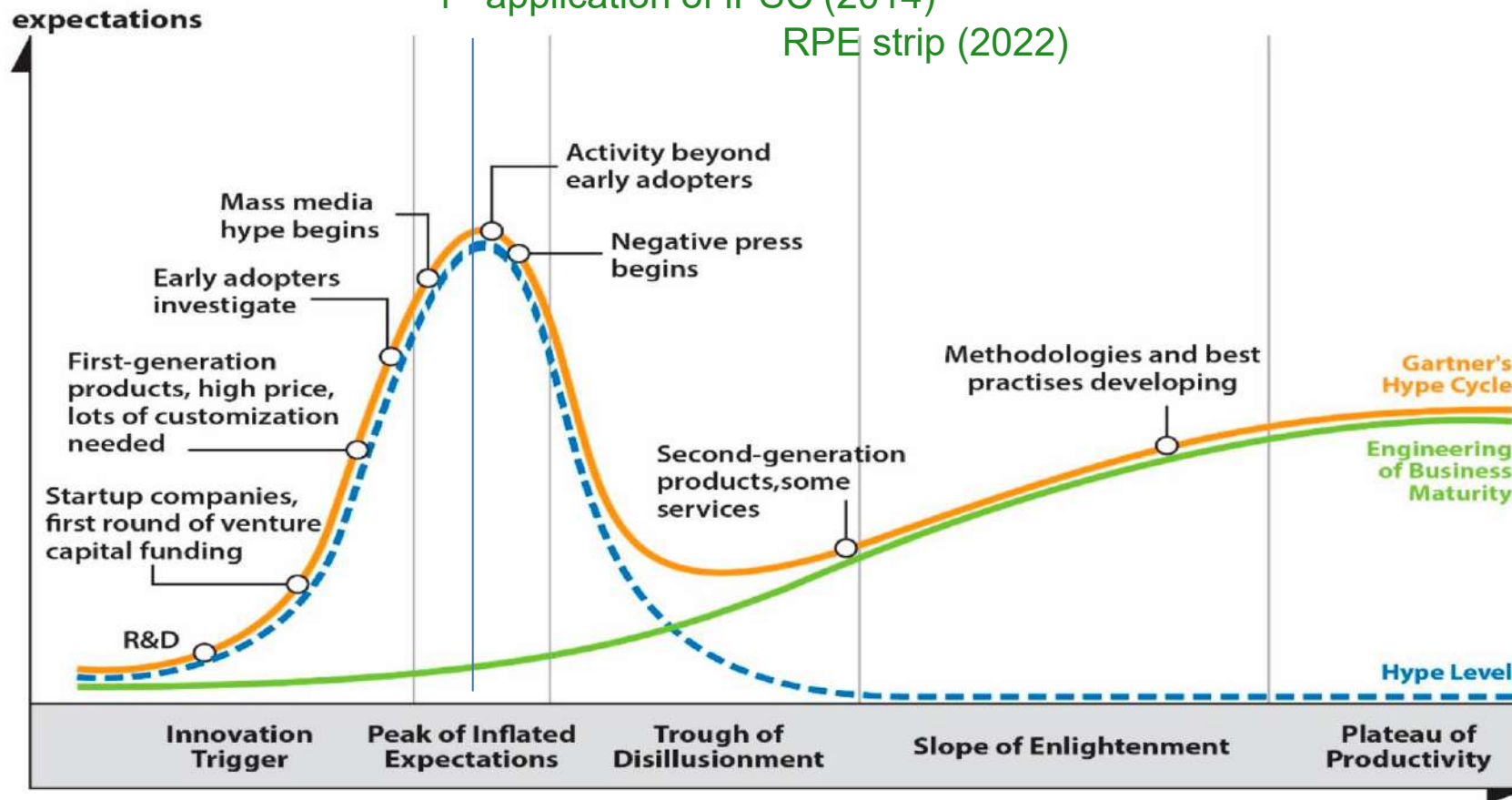
Expectation for new technologies

The Salk Institute Kyoto U Hp RIKEN VCCT

Nobel prize,
Prof. Yamanaka (2012)

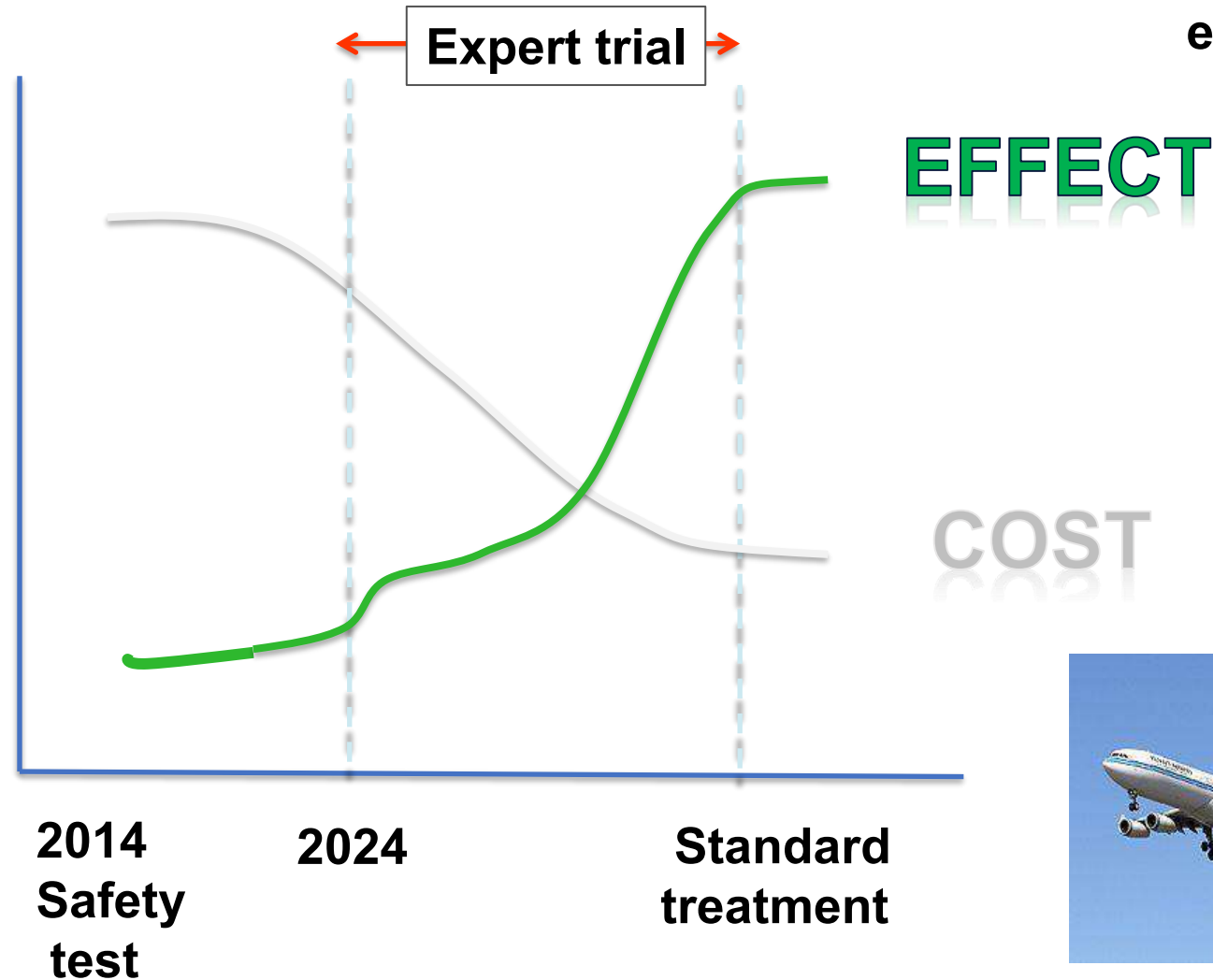
1st application of iPSC (2014)

RPE strip (2022)



Cost vs Benefit of regenerative medicine with **Surgery**

ex) Islet transplantation
Cataract surgery

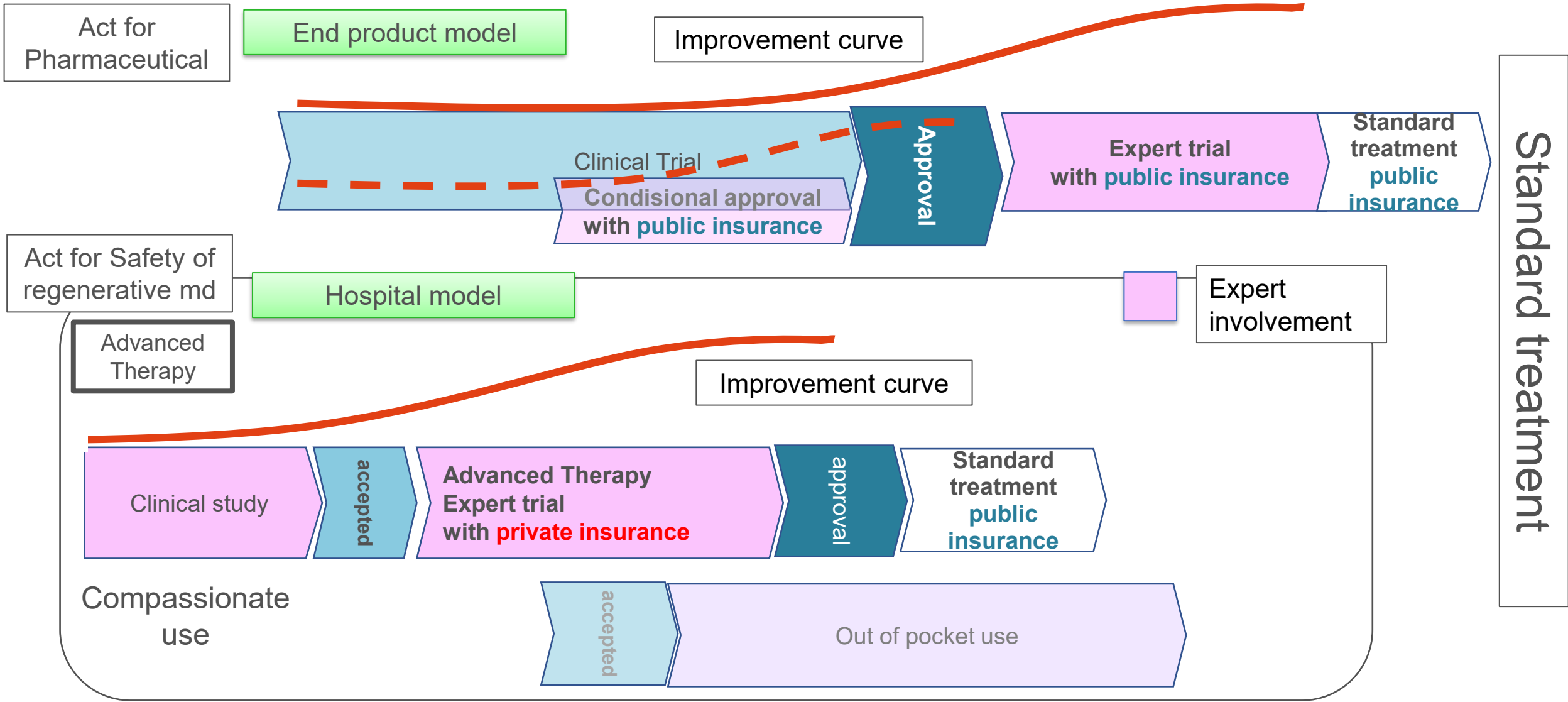


1903



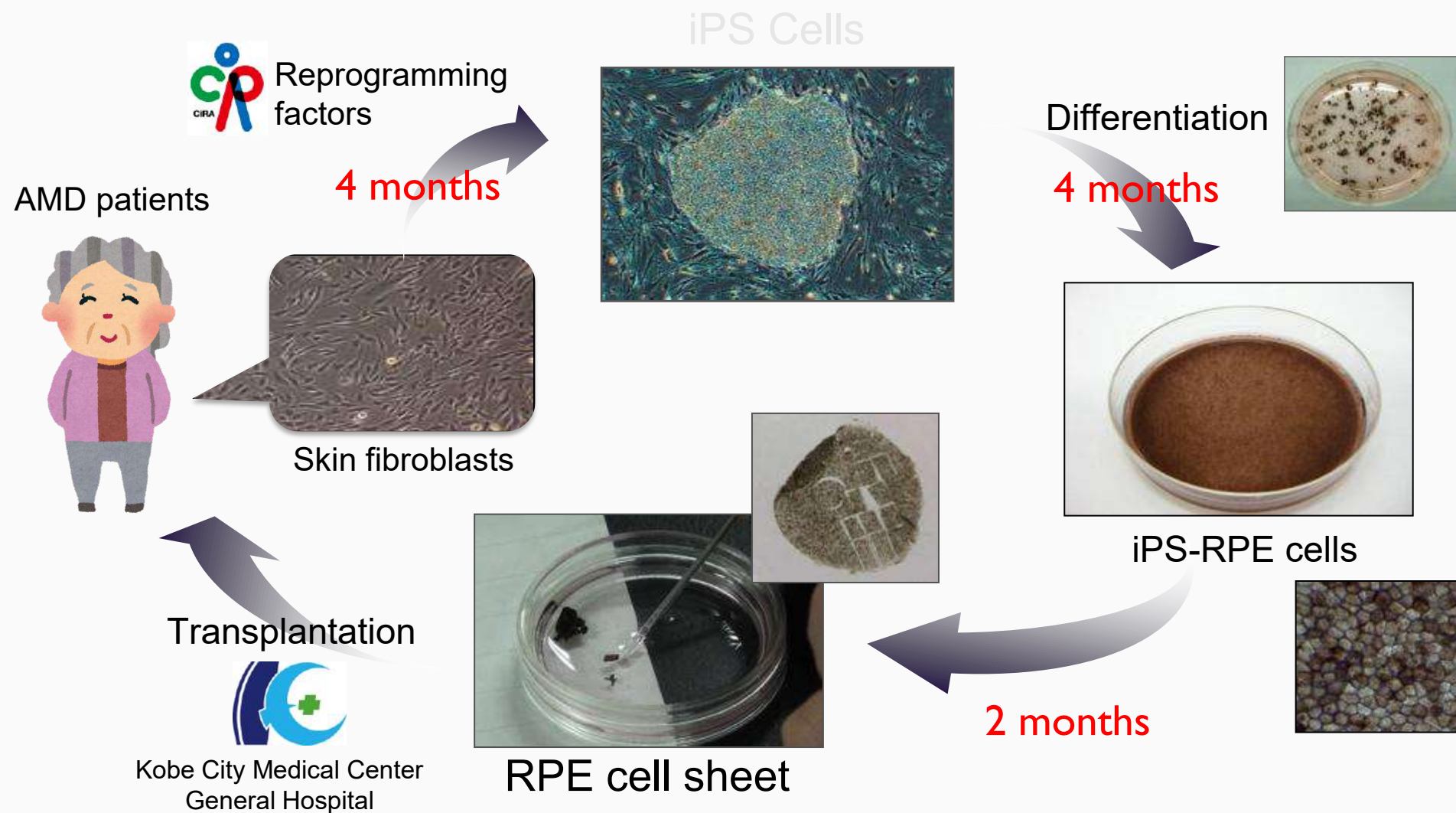
2014~

Various tracks for cell & gene therapy development in Japan



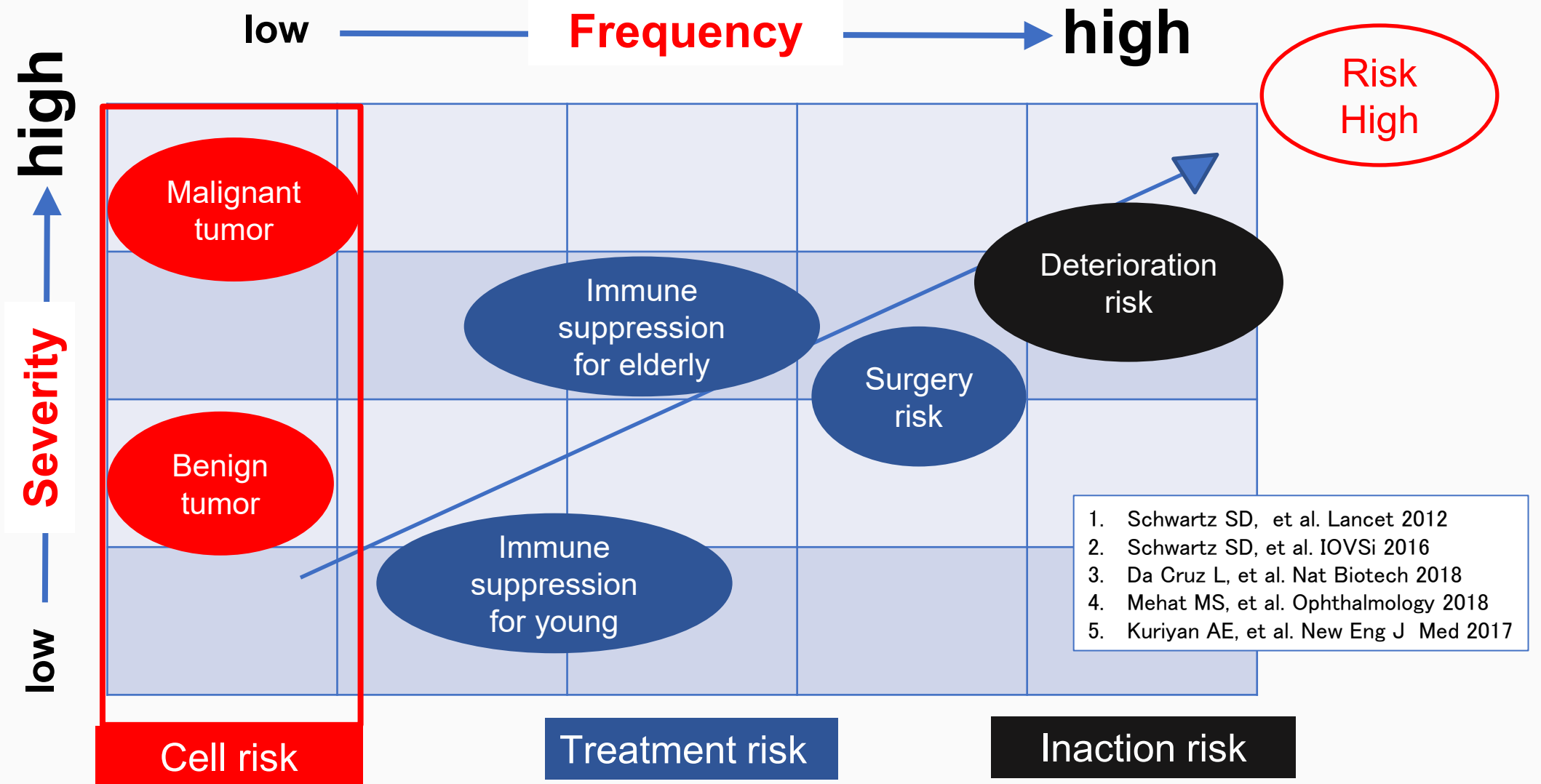
1st clinical research 2013~2015

Autologous iPS-RPE transplantation to AMD patients



Risk Matrix

for iPSC-RPE transplantation (Image)



Strategy for RPE transplantation development

Before the 1st Case

- Determine the Mode of Action – **replacement of RPE** cell layer
- Determine the CQA (Critical Quality Attribute) – **purity**

The 2nd & 3rd clinical study

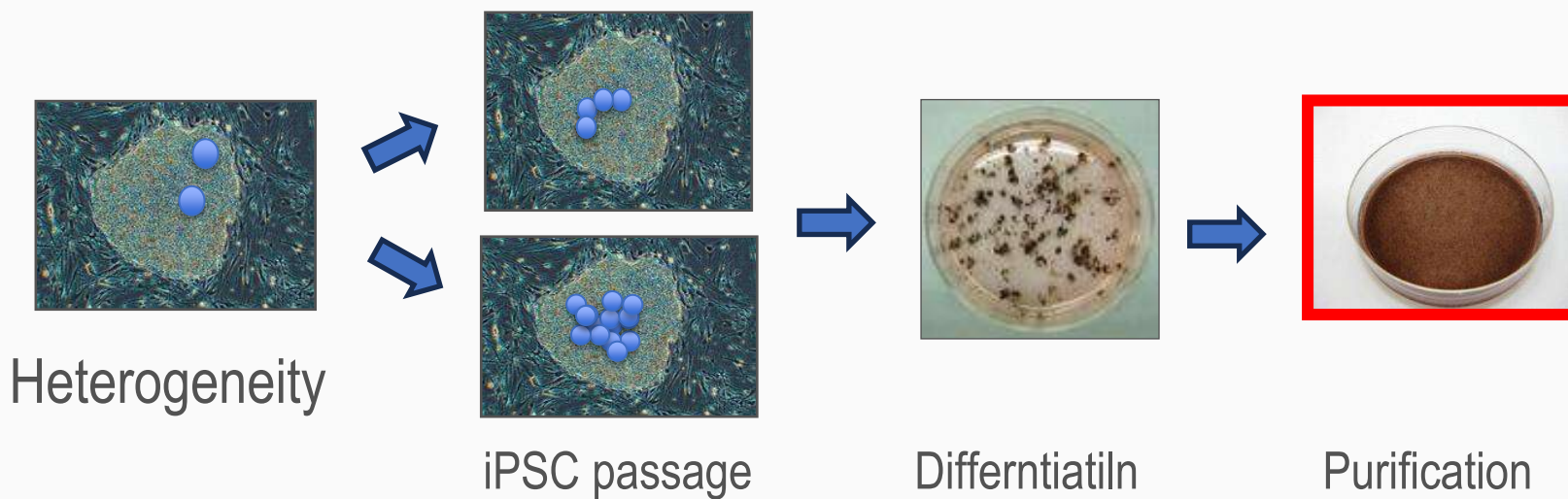
- Select the formulation to solve the risk of surgery – **RPE strip**
- Solve manufacturing skill transfer risks and scale up – using the **humanoid robot**
- Prepare to expand the **surgeons** and hospitals

Clinical trial

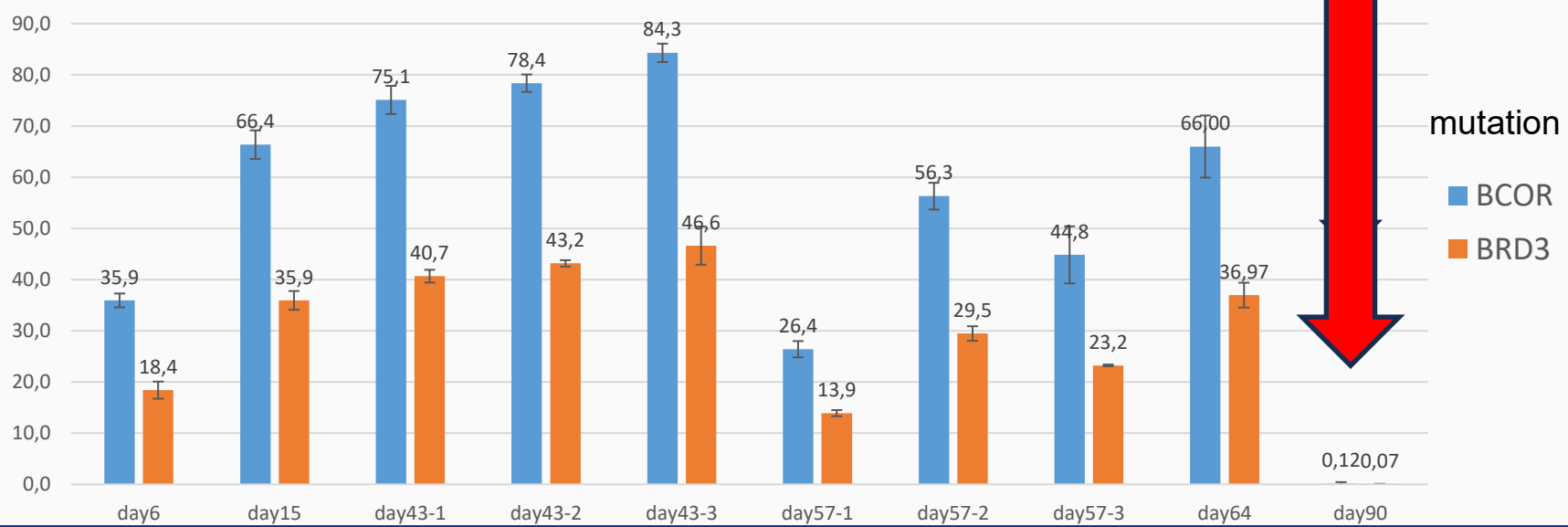
- Avoid the immune suppression risk – Modify the **HLA**
- Do the **clinical trial** with the final product

Heterogeneity of cells in the iPSC colonies

Unpublished data



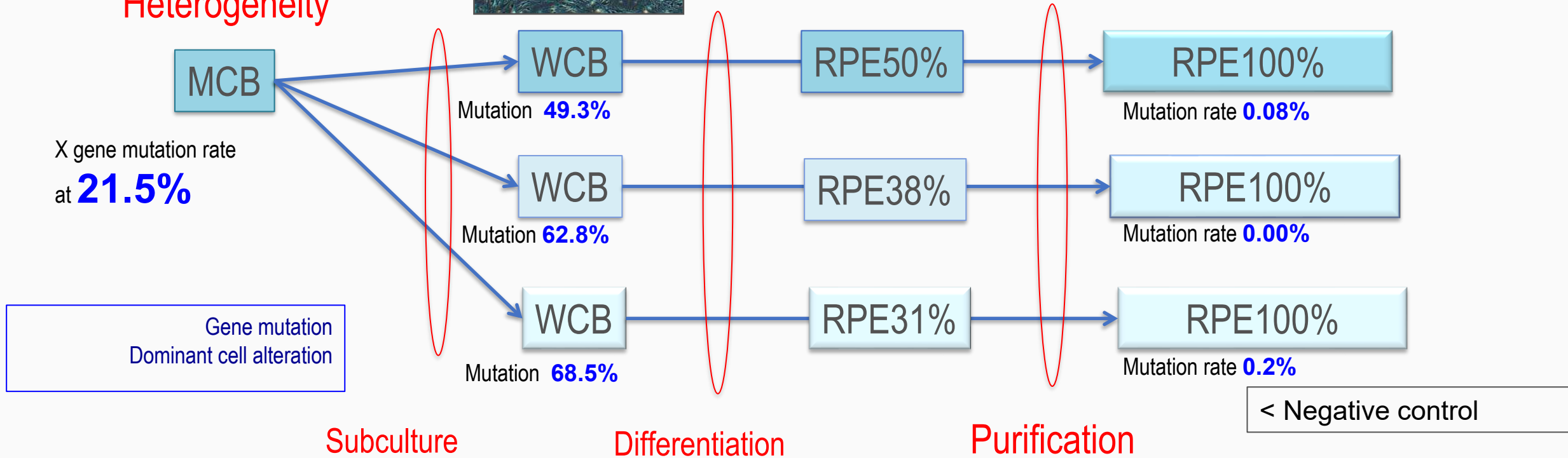
iPS-Ff- line P13 **BCOR, BRD3** mutation ratio time course (n=3)



Heterogeneity between and within the iPSC colonies



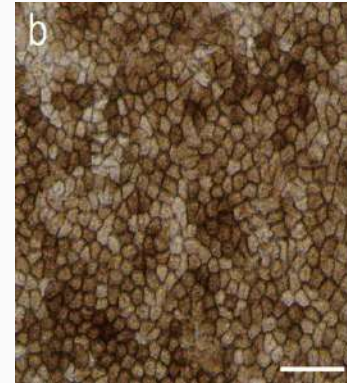
Heterogeneity



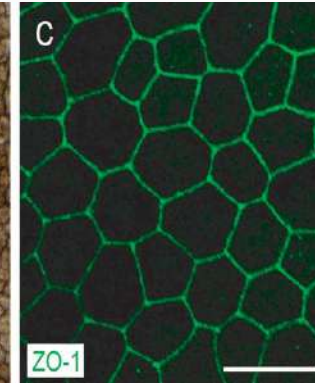
Monitoring points : RPE function

- 1、Phagocytosis
- 2、Trophic factor:
PEDF VEGF
- 3、Barrier formation
- 4、Visual cycle

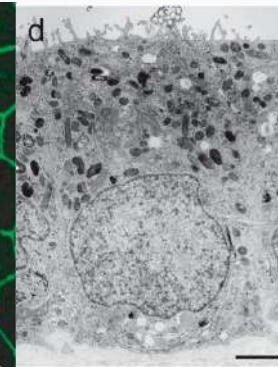
Phlygonal shape



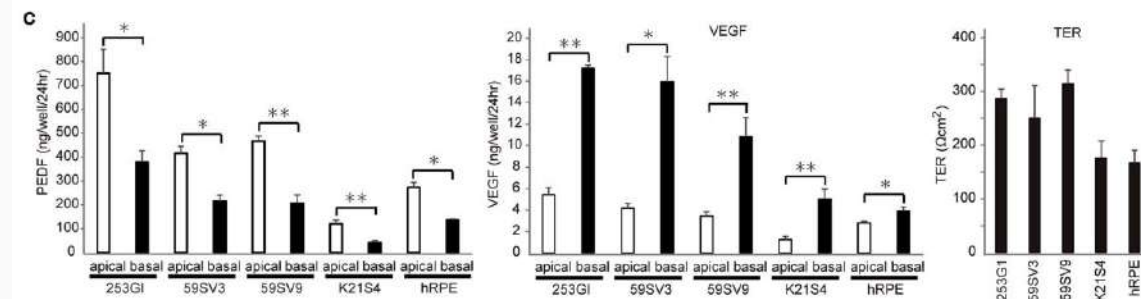
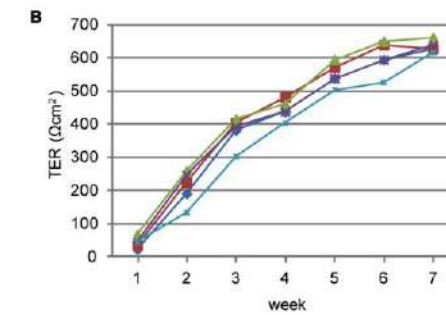
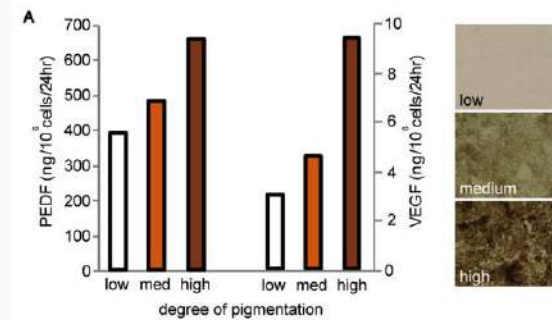
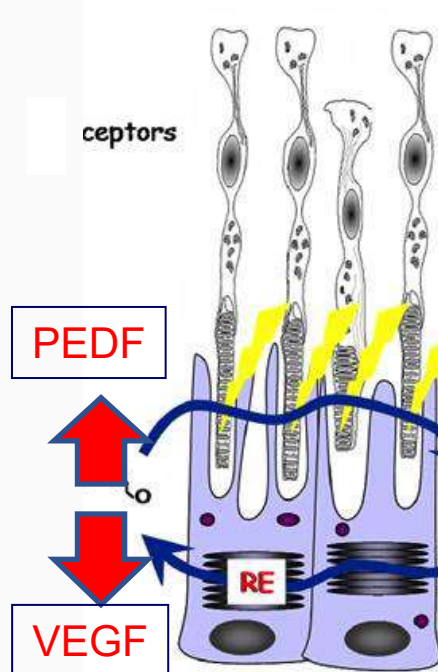
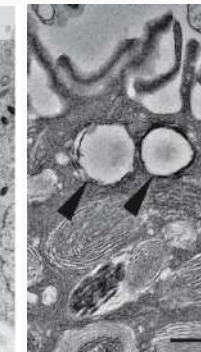
Tight junction



EM



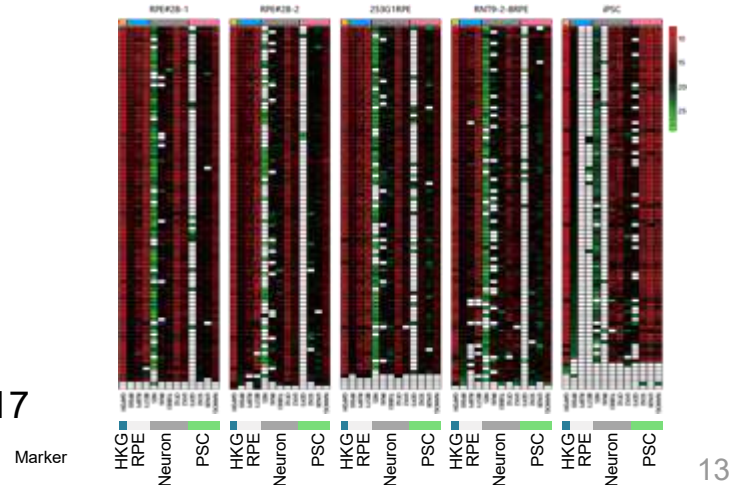
Phago



<Robust protocol > Assessment of purity and equivalence

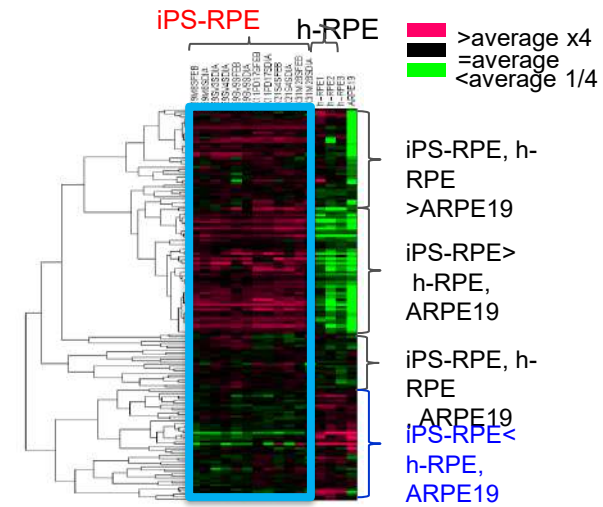


Single cell RT-PCR



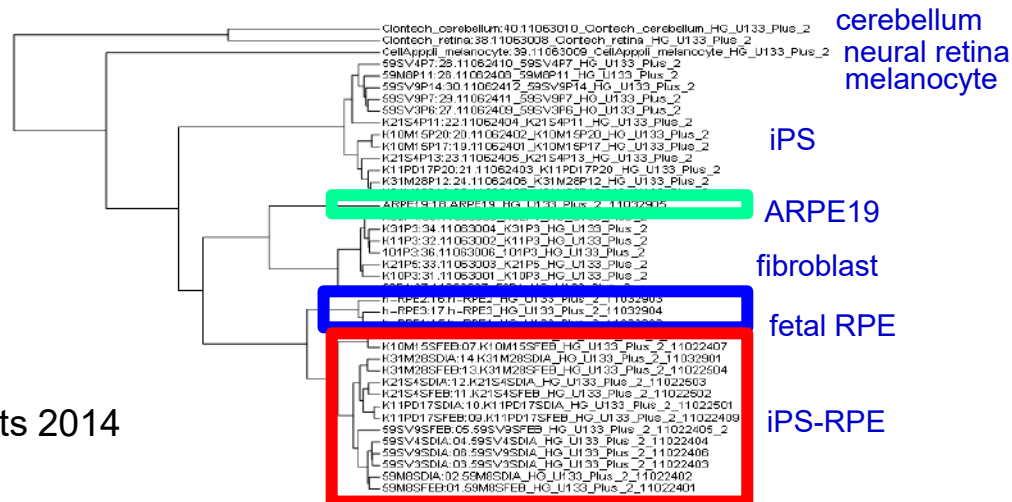
Mandai et al,
New Eng J Med, 2017

RPE signature genes



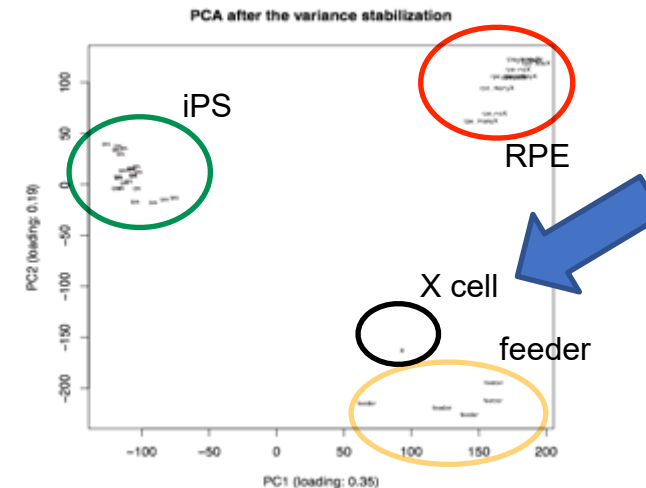
Kamao et al,
Stem Cell Reports 2014

Microarray cluster analysis



Kamao et al,
Stem Cell Reports 2014

Transcriptome analysis



Unpublished data

What are the potentially dangerous cells?

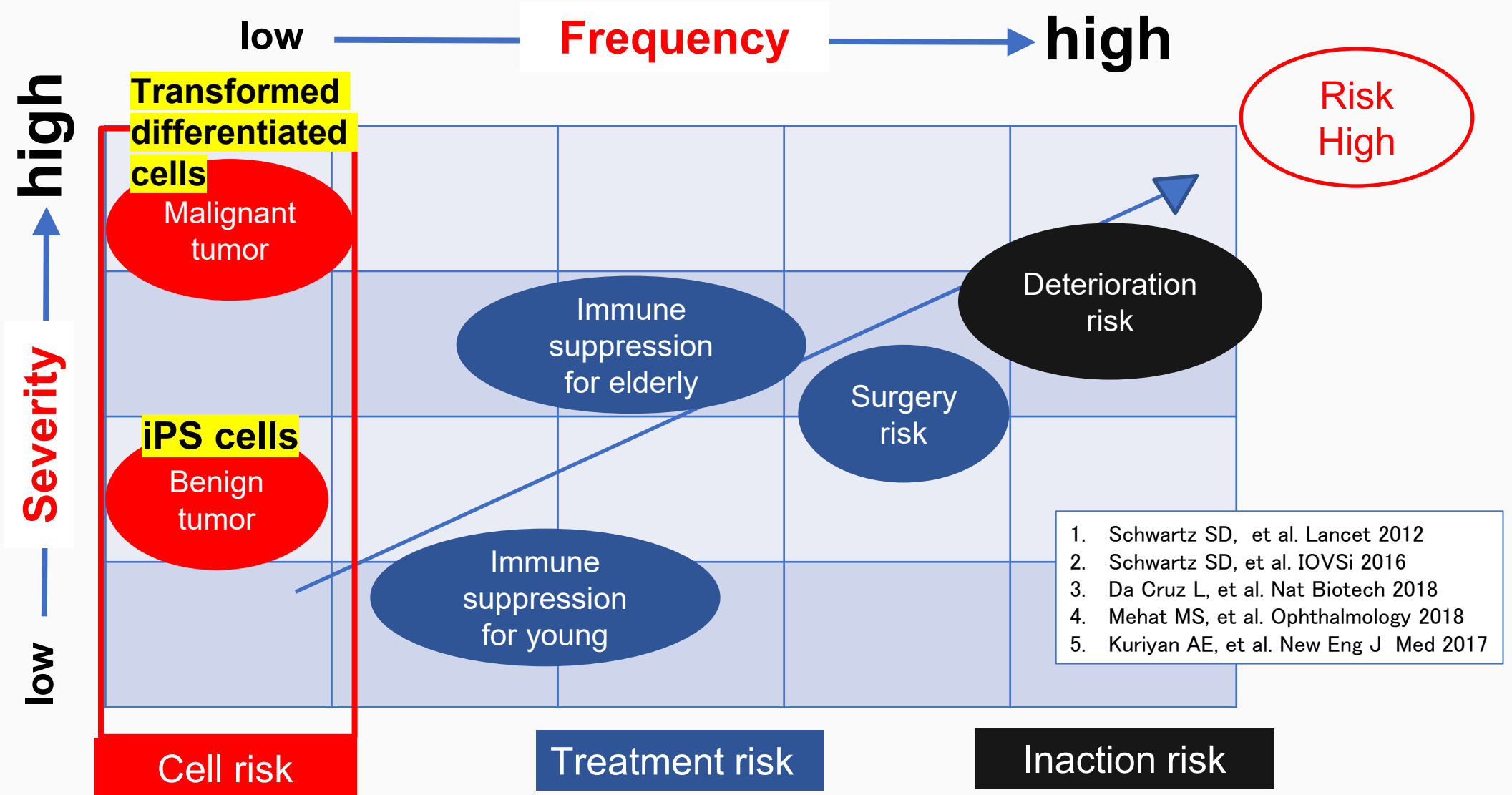
What will happen to patients?

- Residual iPS cells → benign tumor within the eye
 - typically managed with observation per Ophthalmology guidelines (no harm)
- Transformed differentiated cells → malignant tumor → metastatic tumor (life-threatening)
- Oncogene-mutated cells
 - In MSCs or short-lived cells → safe
 - In non dividing cells → no secondary hit → short-term tumorigenicity test (e.g., RPE)
 - In dividing cells → secondary hit may occur → long-term tumorigenicity test or discontinue use

The risky cells are not iPS cells but immature differentiated or transformed cells

Risk Matrix

for iPSC-RPE transplantation (Image)



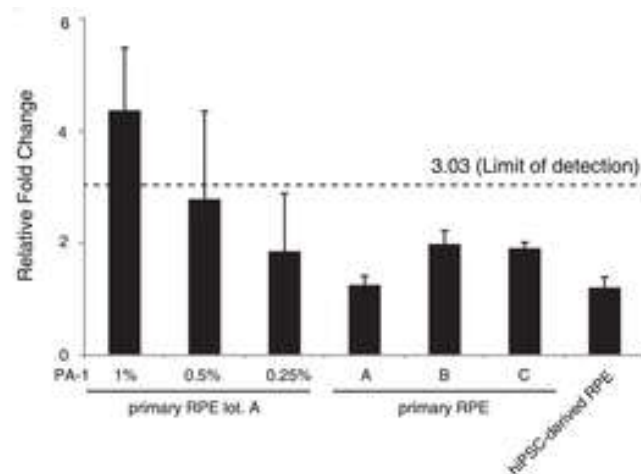
PLoS One. 2012;7(5):e37342. Epub 2012 May 17.

Highly Sensitive In Vitro Methods for Detection of Residual Undifferentiated Cells in Retinal Pigment Epithelial Cells Derived from Human iPS Cells.

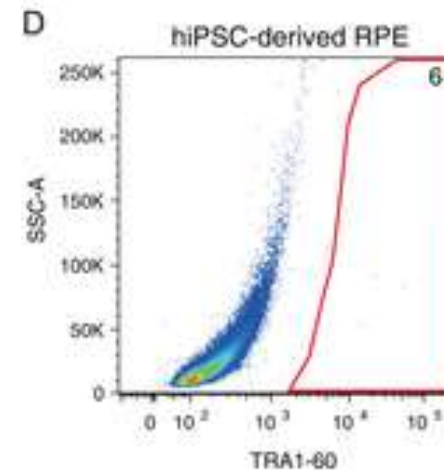
Kuroda T, Yasuda S, Kusakawa S, Hirata N, Kanda Y, Suzuki K, Takahashi M, Nishikawa S, Kawamata S, Sato Y.

National Institute of Health Sciences, Tokyo, Japan

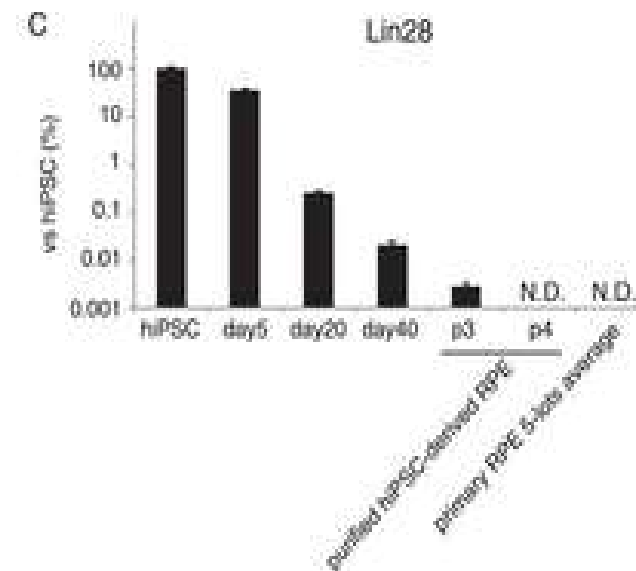
Soft Agar Test



Flowcytometry



qRT-PCR analysis



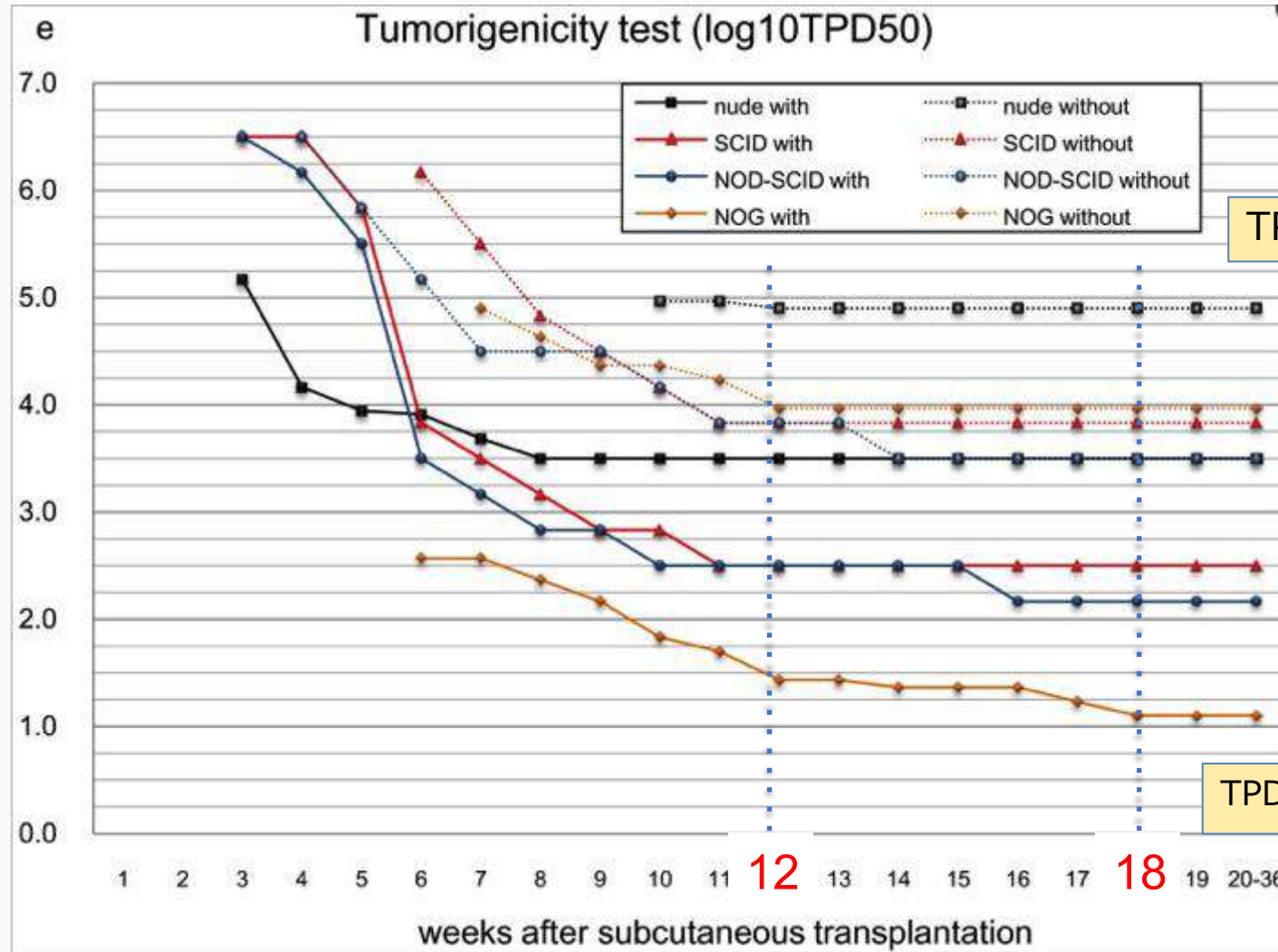
qRT-PCR analysis for Lin28 detects **0.002% of hiPSCs**
a single hiPSC in 5.0×10^4 RPE cells is detectable.

Comparison among immune-deficient mice With or without Matrigel HeLa tumorigenicity test

Kanemura H, Go MJ, Shikamura M, et al PLoS One. 9(1):e85336, 2014



Shin Kawamata



With or w/o
Matrigel

TPD50=Ca 100,000 cells

Nude w/o

NOG w/o
SCID w/o
NOD w/o
nude with

SCID with

NOD with

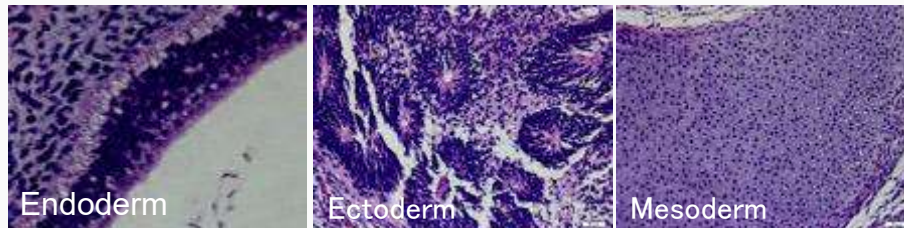
NOG with

TPD50=10^{1.1} (n=75)

Kanemura et al.

Sensitivity of tumorigenicity test (iPS Cells)

- **subcutaneous** (with Matrigel)- NOG mice



iPSC: TPD50 = $10^{2.12}$ (n = 30)

- **subretinal**-(w/o Matrigel)- Nude rat



iPSC: TPD50 = $10^{4.73}$ (n = 20)

Graft cells: iPSC 201B7
observation : 60w~

Summary of tumorigenicity test

(Kanemura et al.2014 PlosONE)

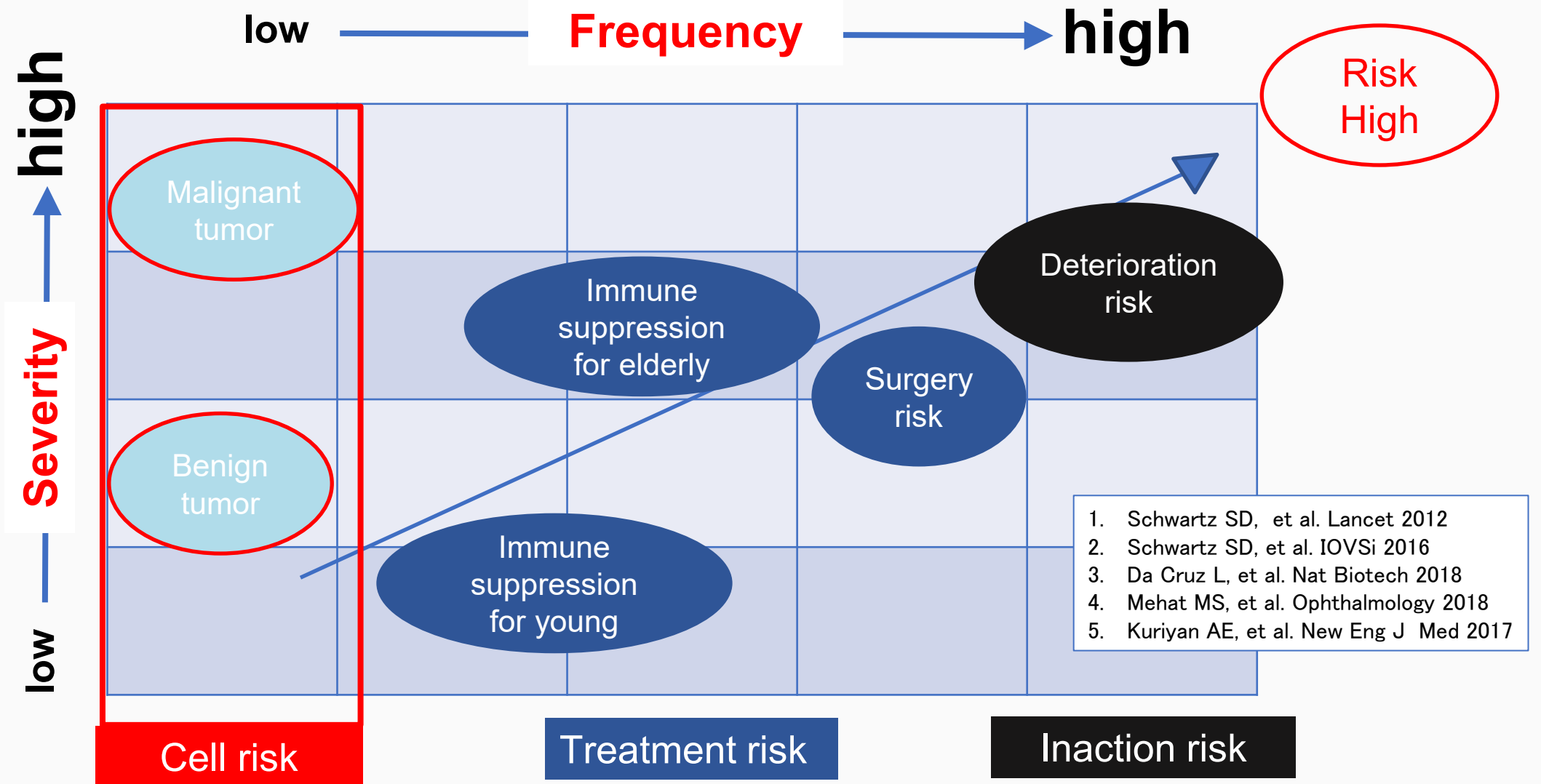
NOD-SCID mice, subcutaneous, 1×10^5 cells in Matrigel

Purified hiPS-RPE did not generate tumors

	donor	Vector	# of animal	tumor
1st 2010. 11~2011. 9	RPE cell	Retrovirus Sendai virus Plasmid A	57	0
2nd 2011. 2~2012 4	RPE cell	Plasmid A	11	0
	RPE cell: OCT3/4 remnant+	Plasmid A	7	0
3rd 2012. 3~10	RPE cell	Plasmid B	27	0
4th 2012. 12~2013 7	RPE sheet (subcutaneous· subretinal)	Plasmid B	16	0

Risk Matrix

for iPSC-RPE transplantation (Image)

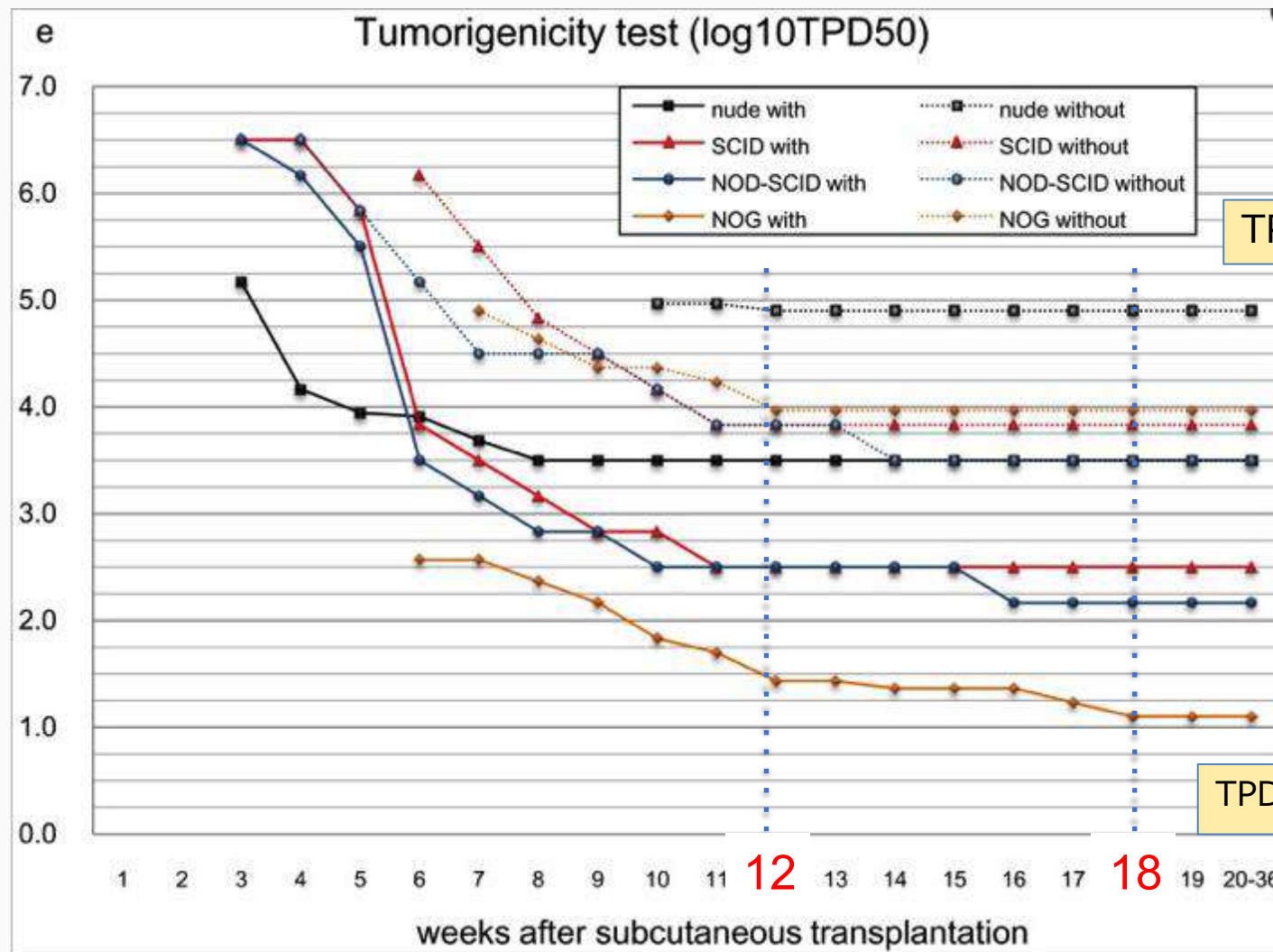


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Shin Kawamata



With or w/o
Matrigel

TPD50=Ca 100,000 cells

Nude w/o

NOG w/o
SCID w/o
NOD w/o
nude with

SCID with

NOD with

NOG with

TPD50=10^{1.1} (n=75)

Kanemura et al.

Summary of tumorigenicity test

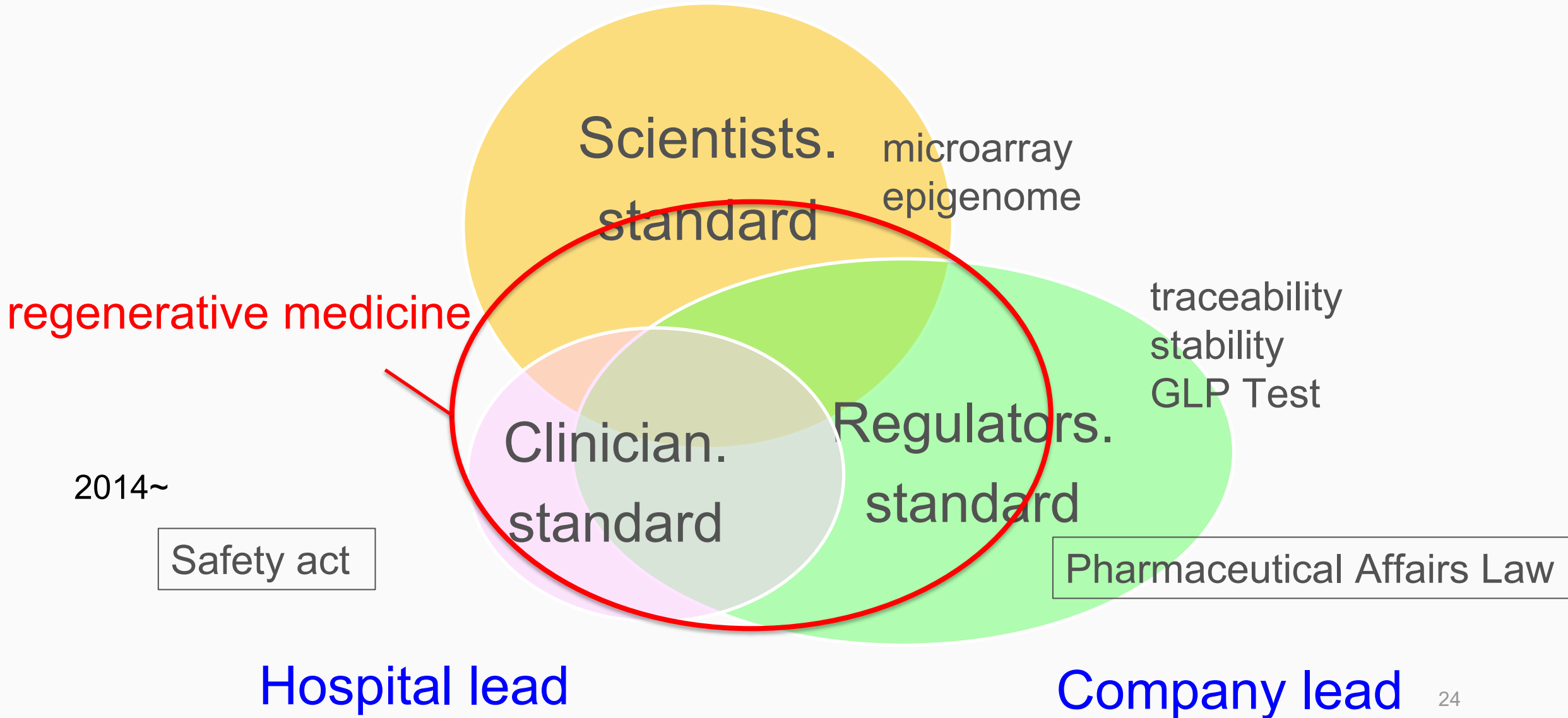
(Kanemura et al.2014 PlosONE)

NOD-SCID mice, subcutaneous, 1×10^5 cells in Matrigel

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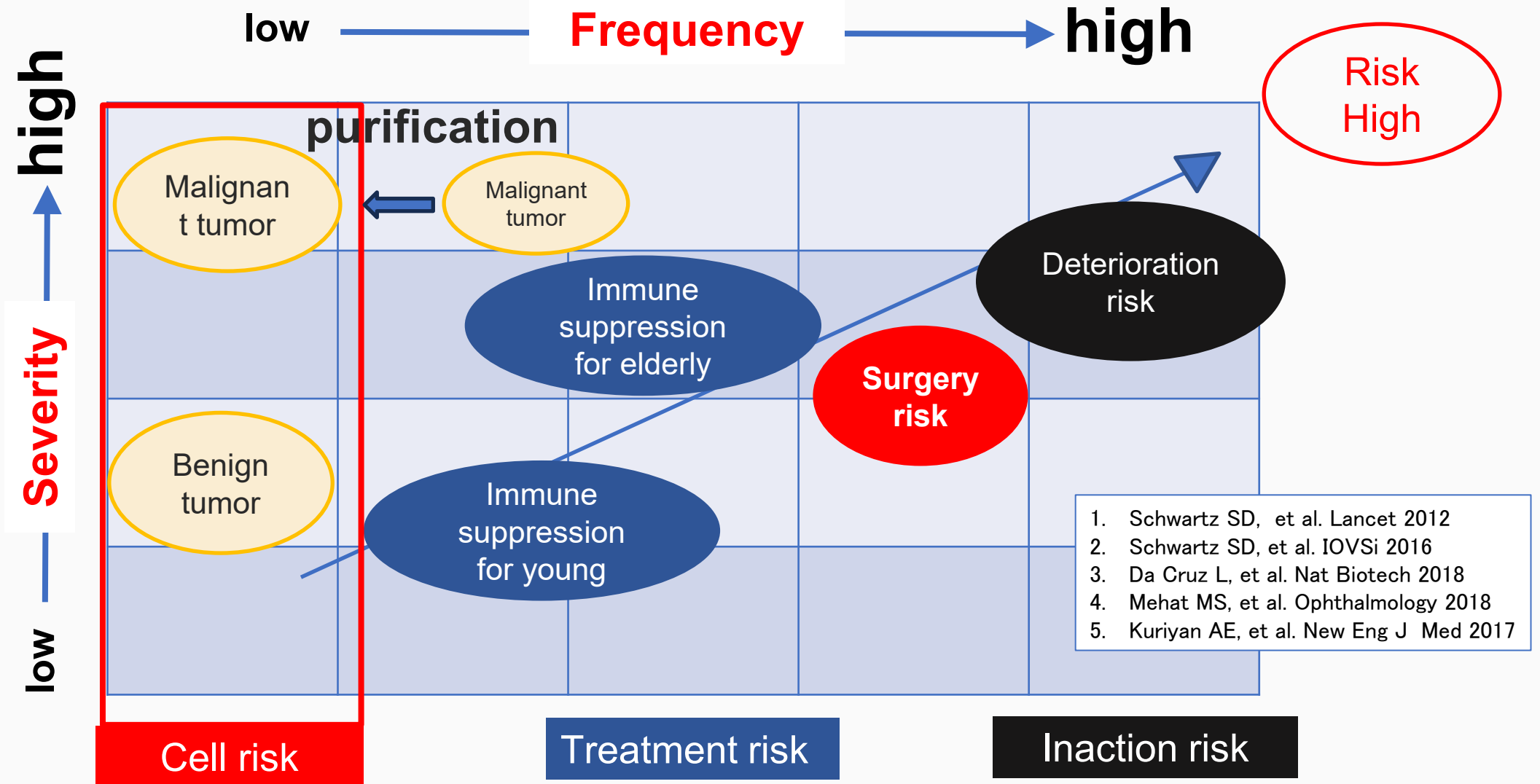
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4th 2012. 12~2013 7	RPE sheet (subcutaneous· subretinal)	Plasmid B	16	0

Convincing data for each field

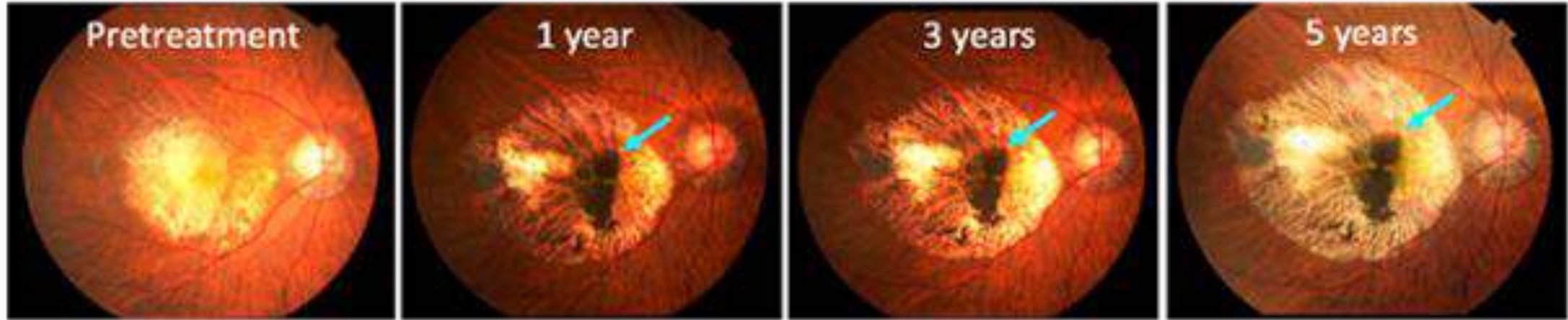


Risk Matrix

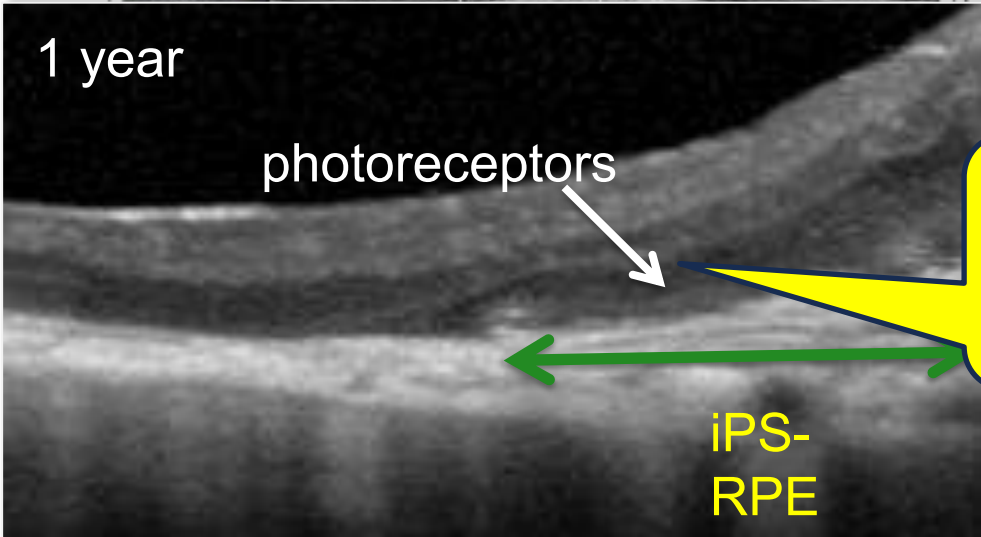
for iPSC-RPE transplantation (Image)



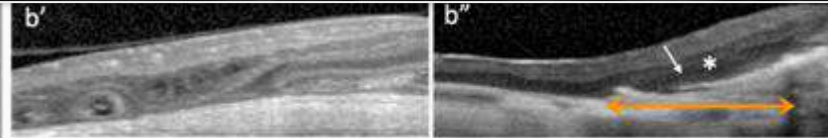
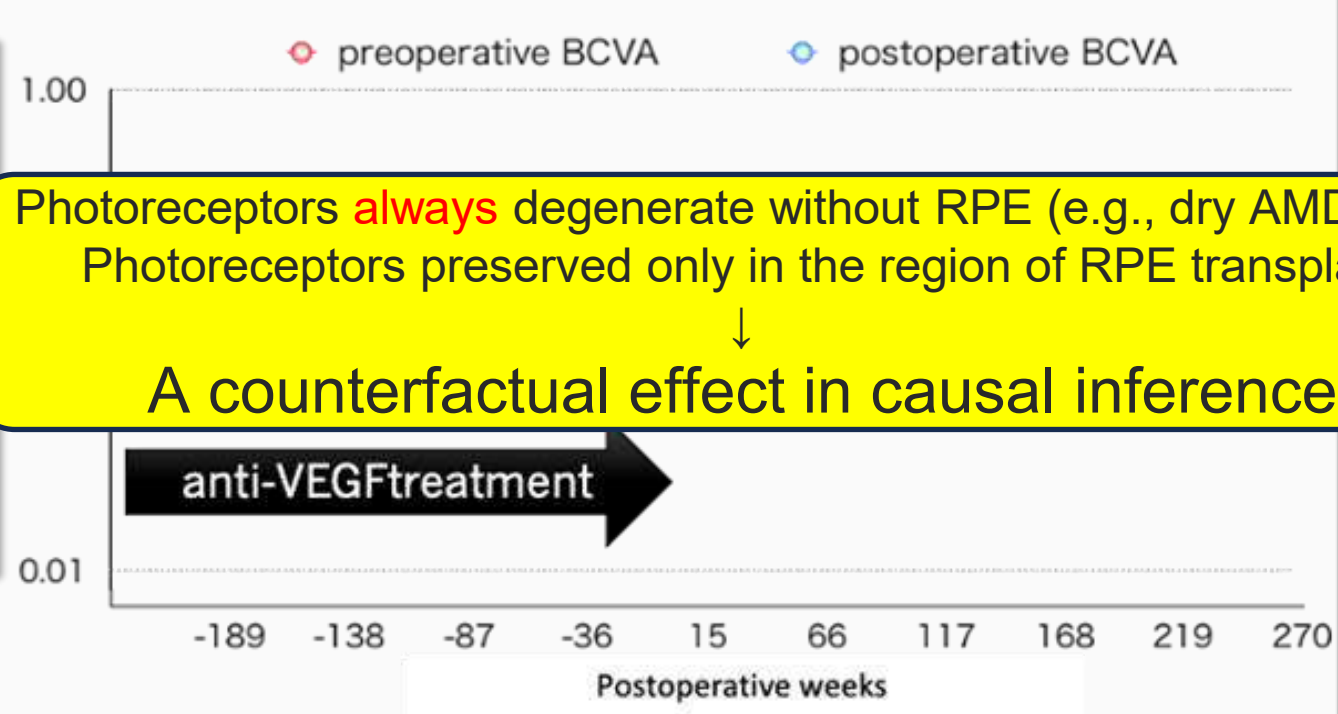
Autologous IPSC-RPE transplatnation from 2014



B OCT images taken before and after surgery



C Visual Acuity measurement before and after surgery



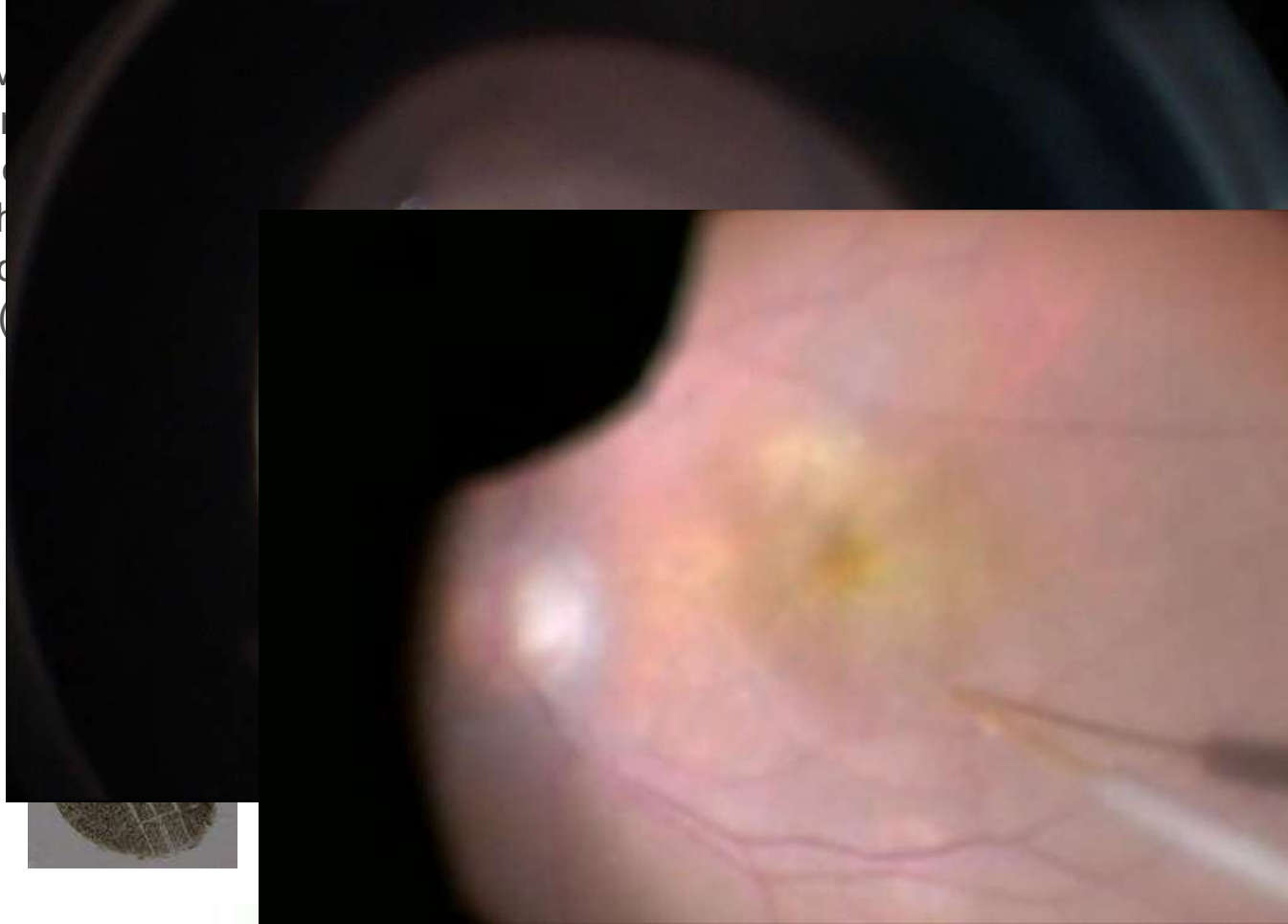
For the standard treatment (2017~) Allogeneic iPSC-RPE transplantation

Totally 10 patients

- No serious adverse events
- No severe surgical complications
- All have long survival
- All patients maintain their vision
- Two of them showed only subjective symptoms (e.g. floaters)

First-in-human

Autologous
iPSC-derived
RPE sheet,
2013.08-2015.09



Final product

HLA partially KO
RPE strip
w/o immune suppression

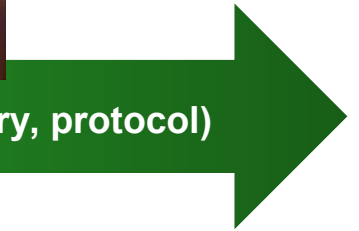


RP

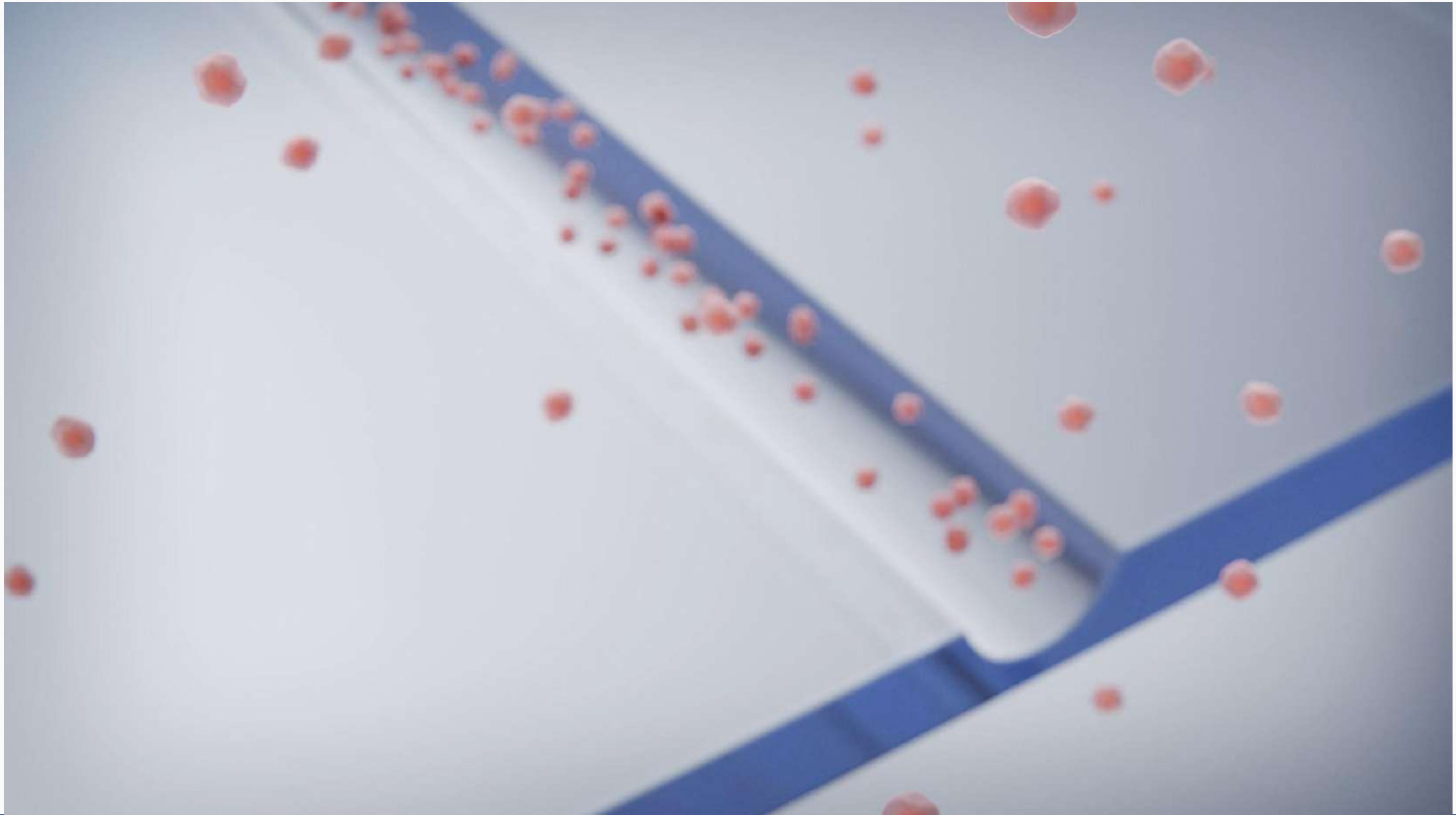


Last 10 years needed to mature technologies (e.g. surgery, protocol)

1 patient of wAMD;



RPE strip transplantation

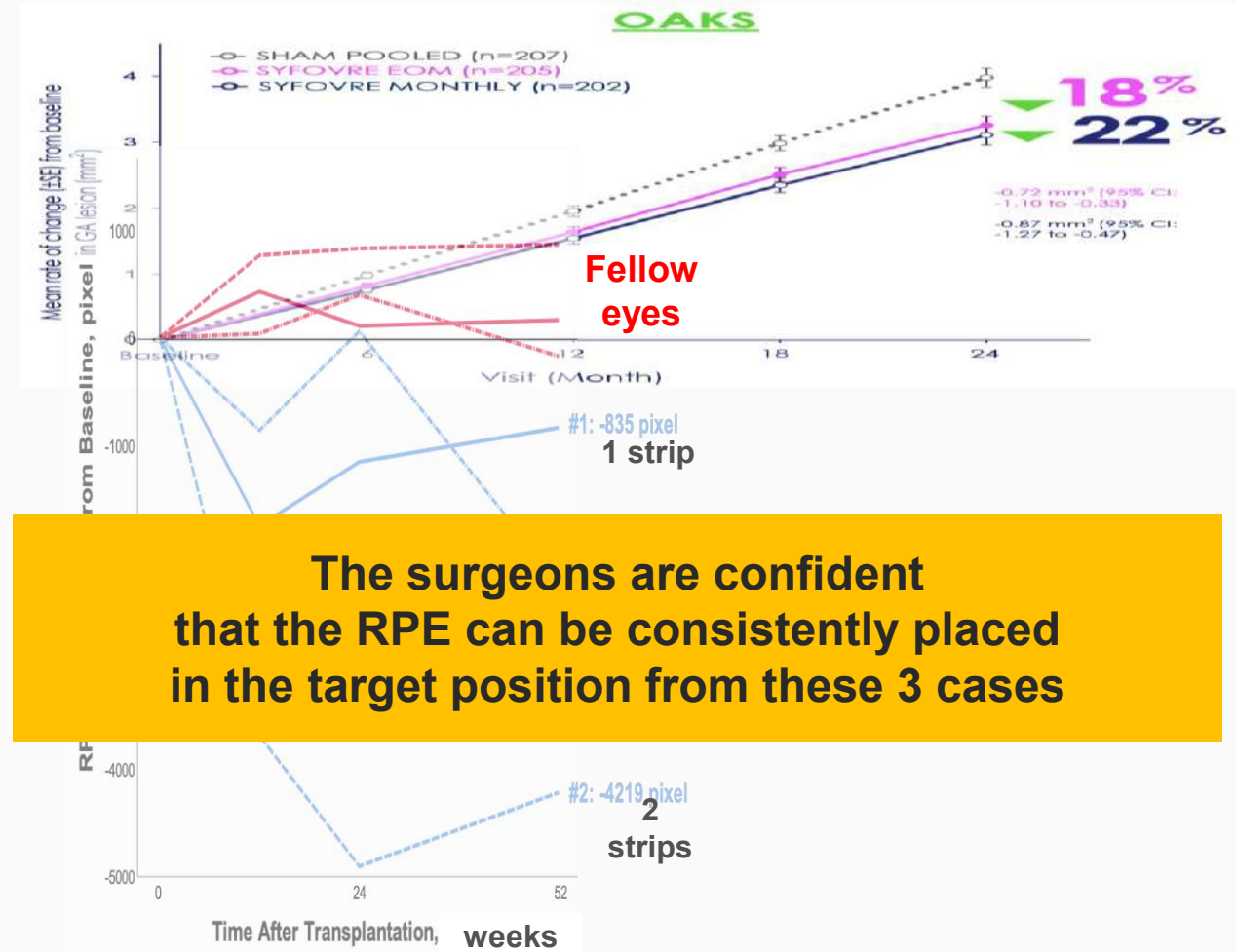


RPE strip Safety & Efficacy (n=3)

Complication

Ocular SAE	RPE strip (n=3)	RPE suspension (n = 5)
ERMembrane (removal ope)	1 (33%) 0	3 (60%) 1 (20%)
Macular edema	1 (33%)	1 (8%)
Subretinal fluid	0	0
Retinal hemorrhage	0	0
RPE detachment	0	0
Neovascularization	0	0
Retinoschysis	0	0
Retinal tear	0	0
Subretinal fibrosis	0	0
Macular hole	0	1

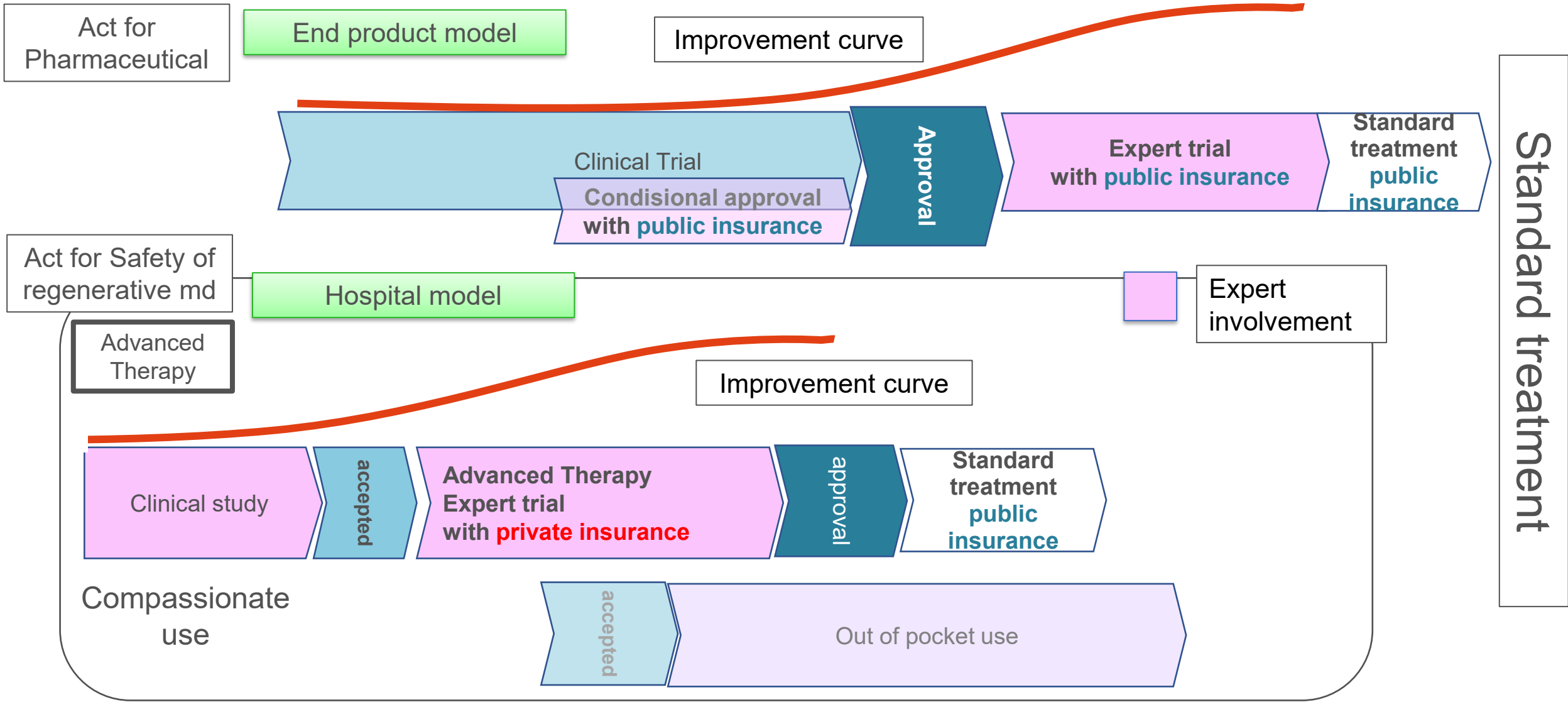
Red line: Most severe adverse event



The surgeons are confident that the RPE can be consistently placed in the target position from these 3 cases

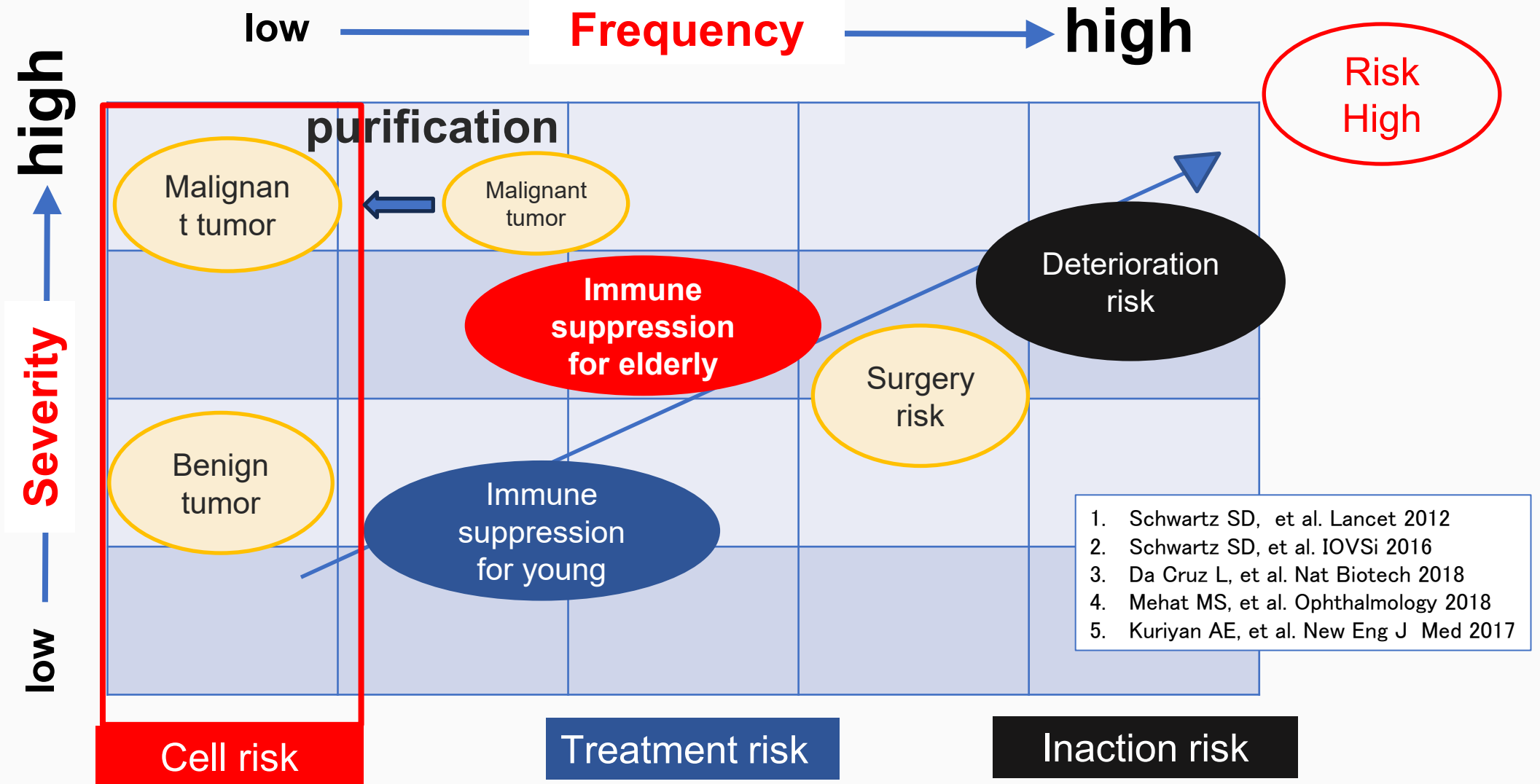
2014~

Various tracks for cell & gene therapy development in Japan



Risk Matrix

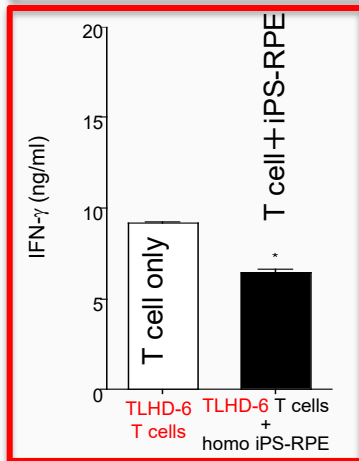
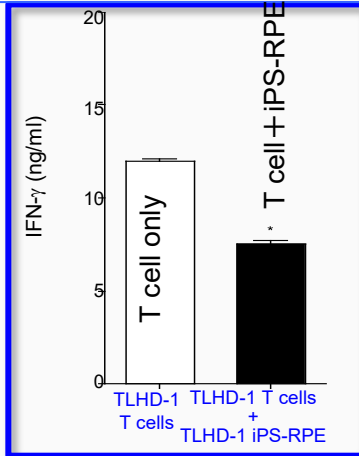
for iPSC-RPE transplantation (Image)



(In vitro) HLA-DR matched allogeneic iPS-RPE suppress T cell activity as well as autologous transplantation

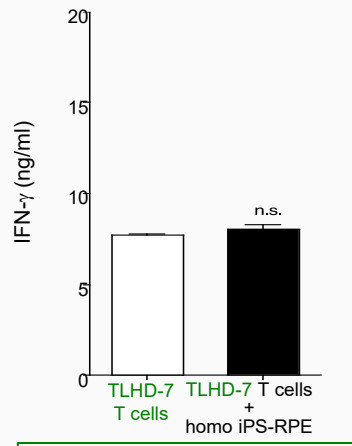
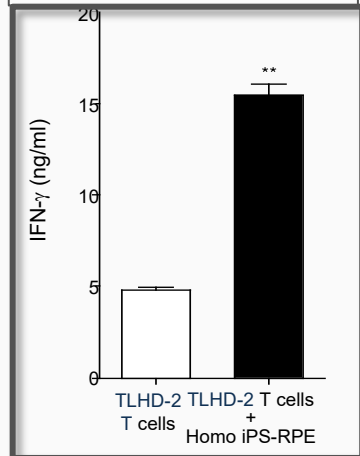
(Sugita et al. Stem Cell Reports 2016)

Autologous iPS-RPE



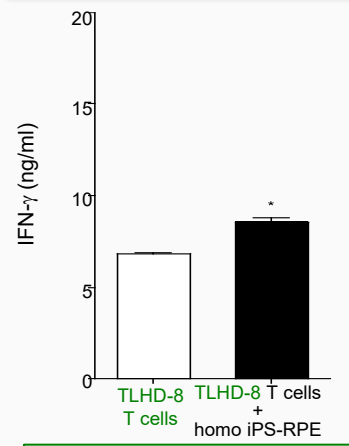
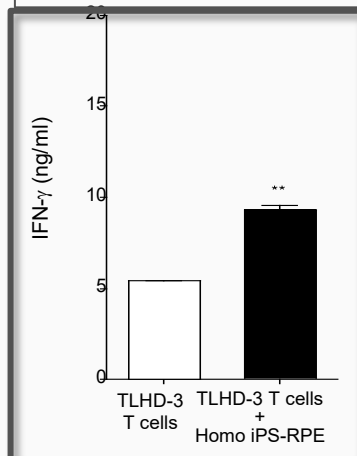
Allo DR match

HLA mismatch



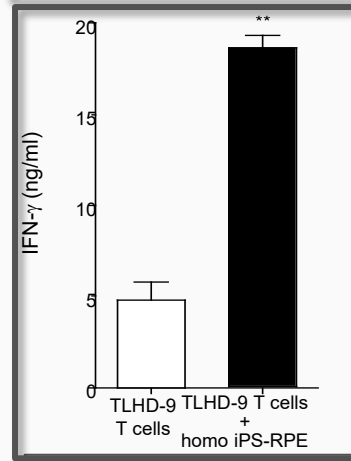
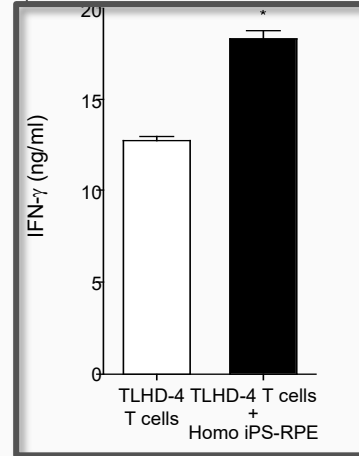
DR haplo match

HLA mismatch



DR haplo match

HLA mismatch



HLA mismatch

T cells

TLHD-1 : DR9/DR16 Auto

TLHD-2 : DR4/DR13

TLHD-3 : DR4/DR8

TLHD-4 : DR4/-

TLHD-6 : DR8/DR15

(DRB1*15:02)

DR match

TLHD-7 : DR14/DR15

(DRB1*15:01) DR

haplomatch

TLHD-8 : DR12/DR15

(DRB1*15:01) DR

haplomatch

TLHD-9 : DR4/DR12

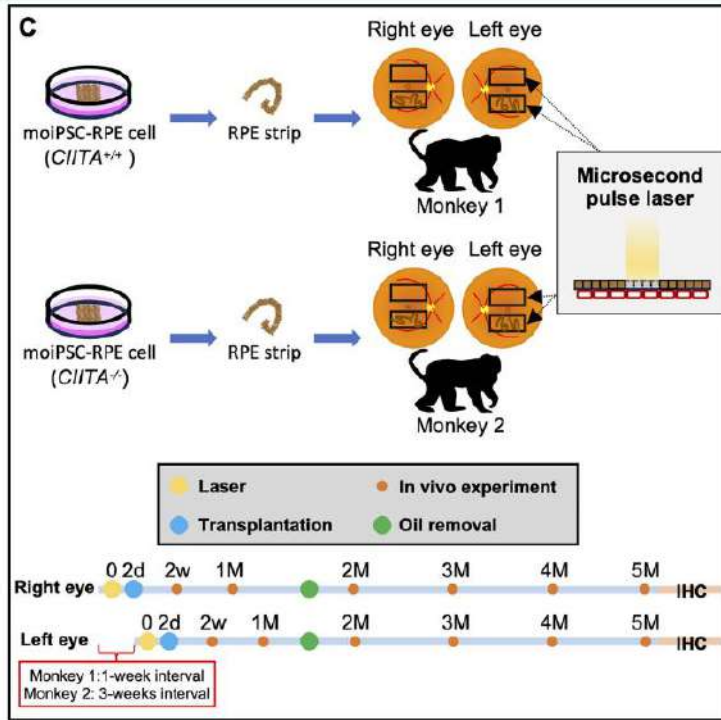
iPS

Homo (454E2) iPS:

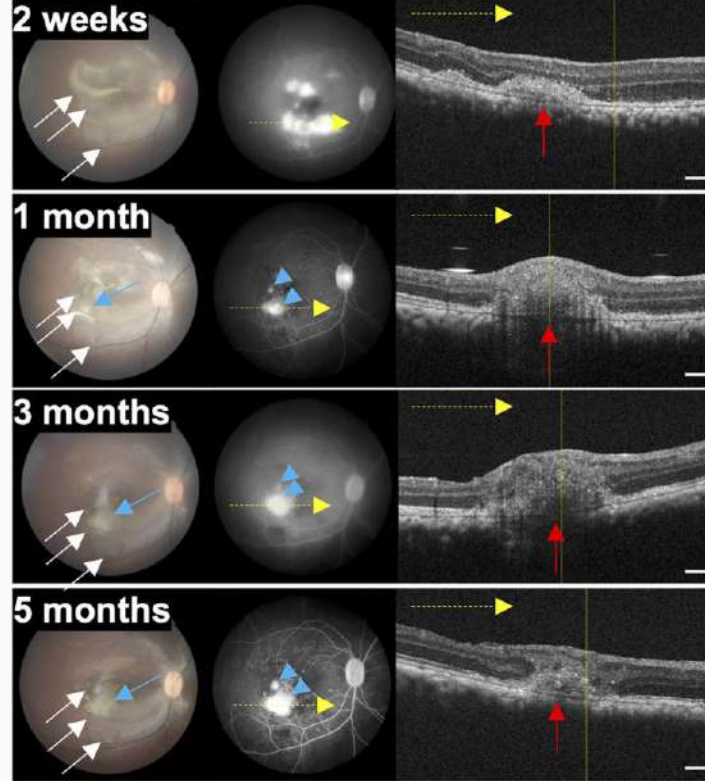
DR15/- (DRB1*15:02)

* $P < 0.05$, ** $P < 0.005$
n.s. – not significant

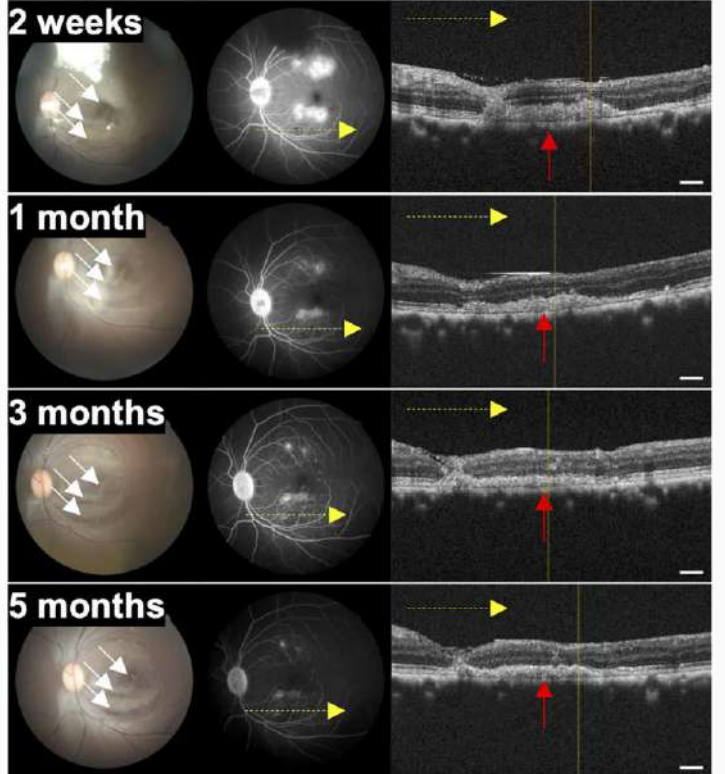
Allogeneic Transplantation of RPE Strips Lacking MHC Class II Can Avoid Rejection in Nonhuman Primate Eyes (Ozaki et al. IOVS Invest. Ophthalmol. Vis. Sci. 2025)



A *CIITA*^{+/+} RPE strips, Right eye

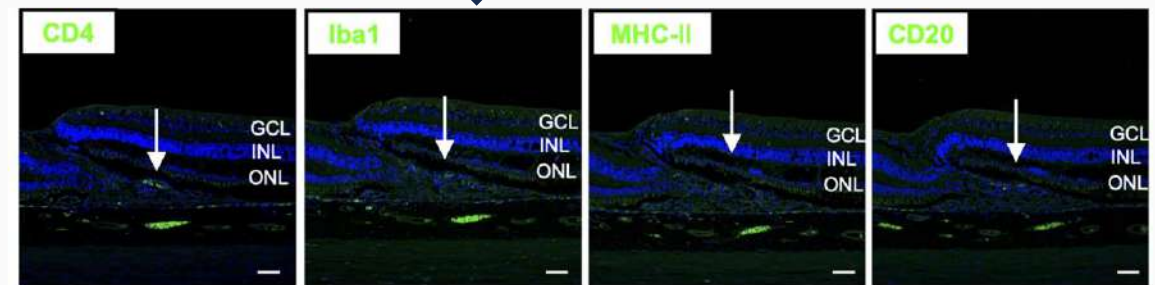
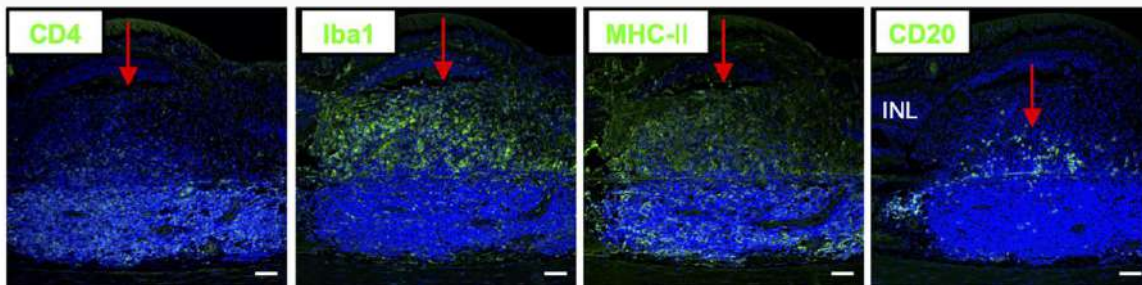


D *CIITA*^{-/-} RPE strips, Left eye



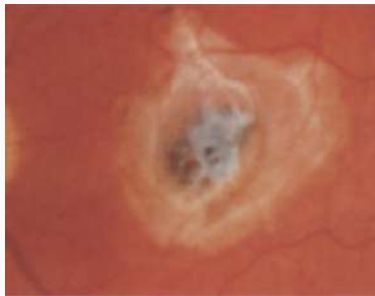
CIITA^{+/+} RPE

CIITA^{+/+} RPE



RPE & photoreceptor cells HLA expression

RPE allograft



rejected
in Monkey

	HLA class 1	HLA class 2
RPE normal	+	-
RPE inflammation	+	+
Photoreceptor normal	+	-
Photoreceptor inflammation	+	-

Virus infection

Immune deficiency

For the standard treatment (2017~) Allogeneic iPSC-RPE transplantation

Totally 10 patients

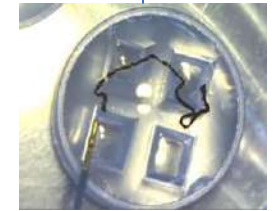
- No serious adverse event
- No severe surgical complication
- All have long survival of the grafts
- All patients maintain their visual function (efficacy)
- Two of them showed clear improvement of subjective symptoms (dAMD & RP)

HLA 6 loci match
w/o immune suppression

Final product

HLA partially KO
RPE strip
w/o immune suppression

with immune suppression
Allogeneic
iPSC-derived
RPE strip
2022 - 2023



First-in-human

Autologous
iPSC-derived
RPE sheet,
2013.08-2015.09



Allogeneic
iPSC-derived
RPE cell suspension
2017.03-2018.09



6 patients of wAMD

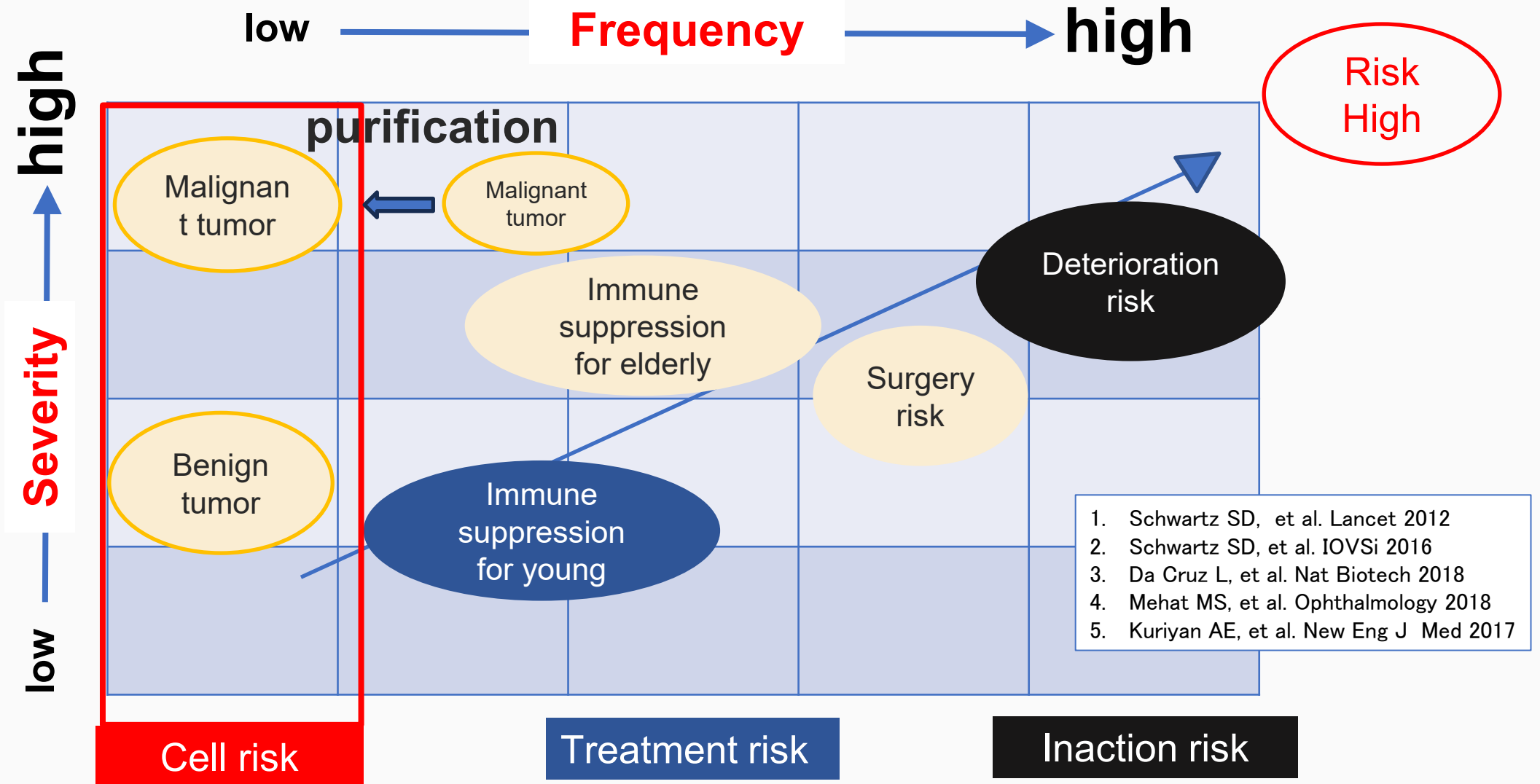
3 patients of dAMD & RP

Last 10 years needed to mature technologies (e.g. surgery, protocol)

1 patient of wAMD;

Risk Matrix

for iPSC-RPE transplantation (Image)



Difference of the Strategies

RPE

Photoreceptor

Before the 1st Case

- Determine the Mode of Action – **replacement of RPE** cell layer
- Determine the CQA – **purity**

The 2nd & 3rd clinical study

- Select the formulation to solve the risk of surgery – **RPE strip**
- Solve manufacturing skill transfer risks and scale up – using the **humanoid robot**
- Expand the **surgeons** and hospitals

Before a clinical trial

- The immune suppression risk – Modify the **HLA**

Before the 1st clinical study

- Determine the Mode of Action – **replacement of photoreceptor** cell layer
- Determine the formulation – **retinal organoid**

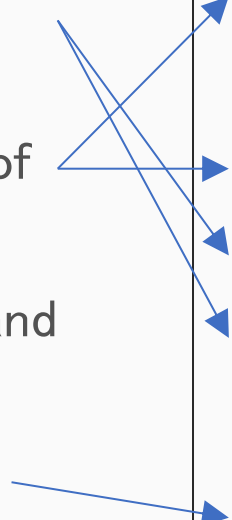
The 2nd clinical study

- Improve the efficacy – **islet-1 KO** iPS organoid
- Determine the CQA – **qPCR, morphology**
- Select the suitable iPS cell lines – **animal model** & electrophysiology
- Expand the target cases – **RPE & photoreceptor combined treatment**

Not necessary

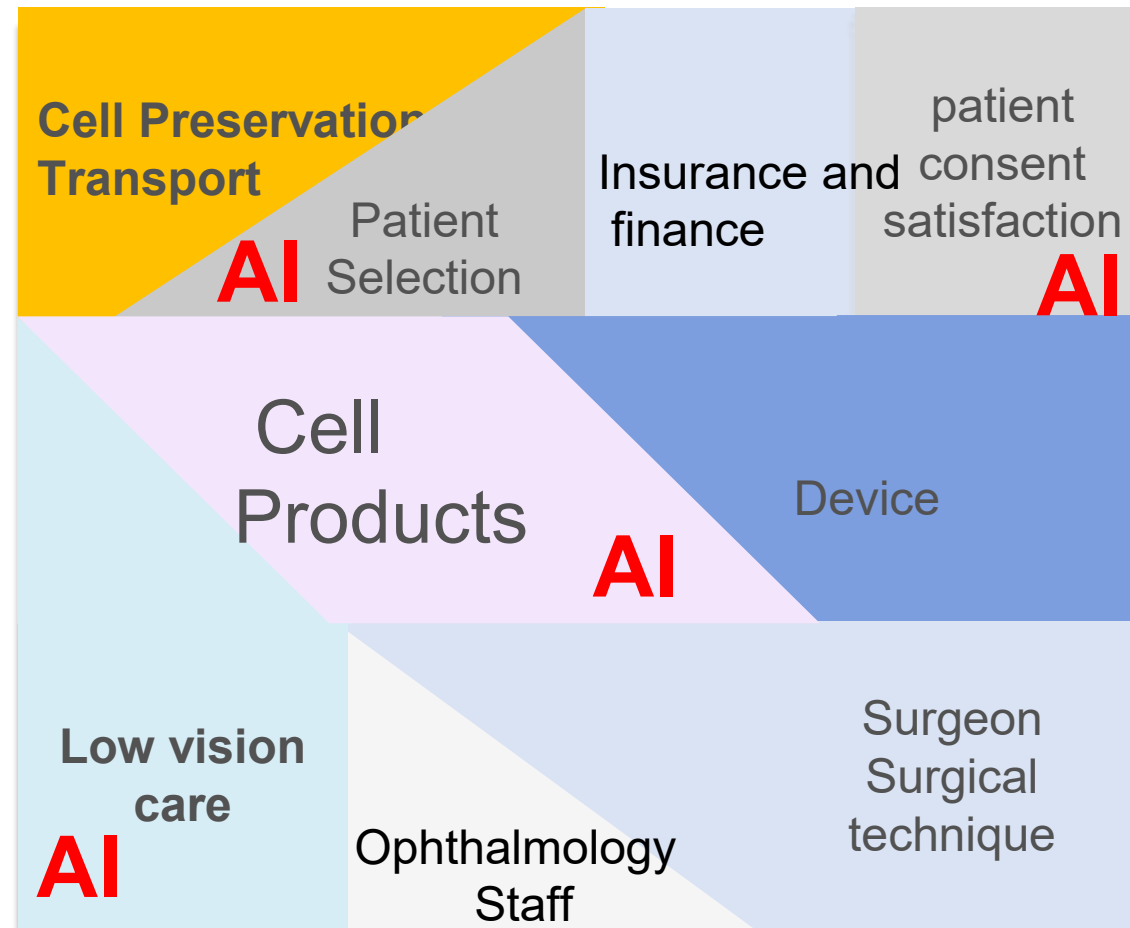


Start the **clinical trial** with the final product

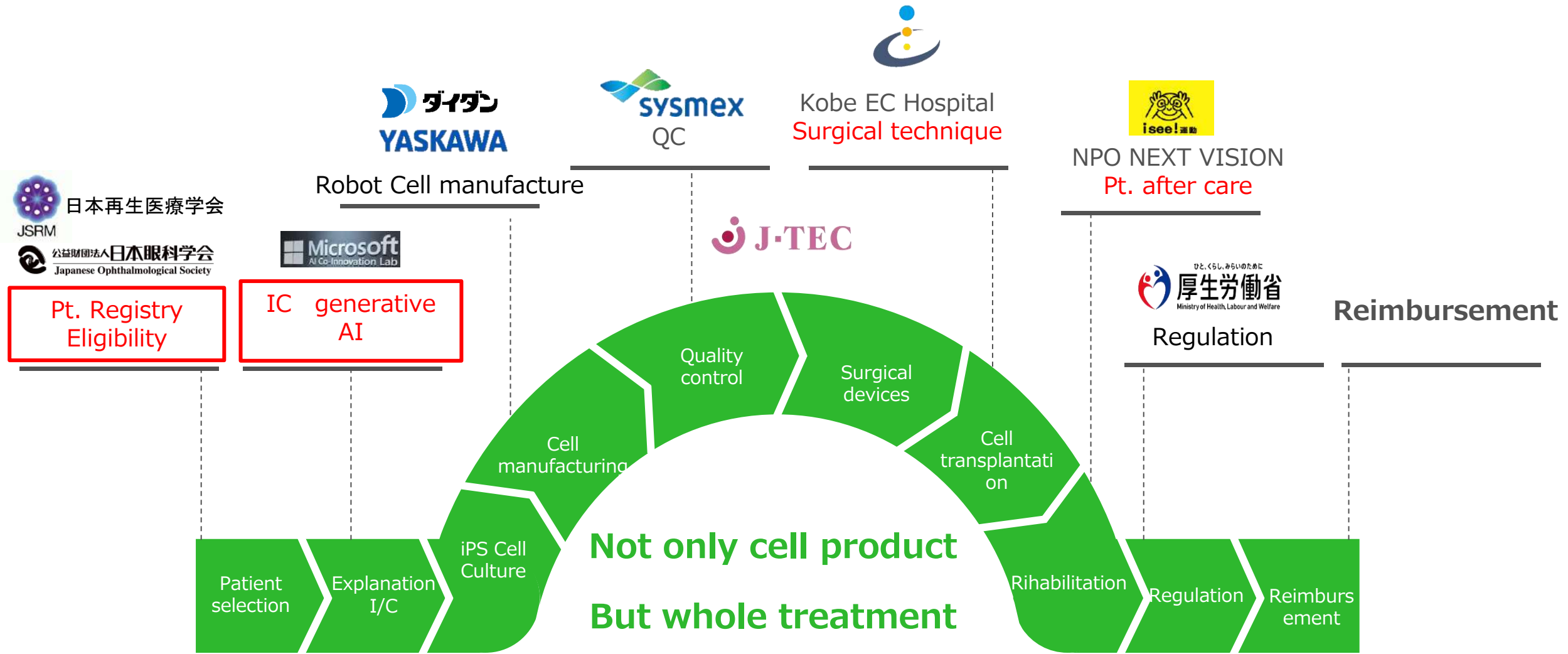


Factors in Regenerative Medicine

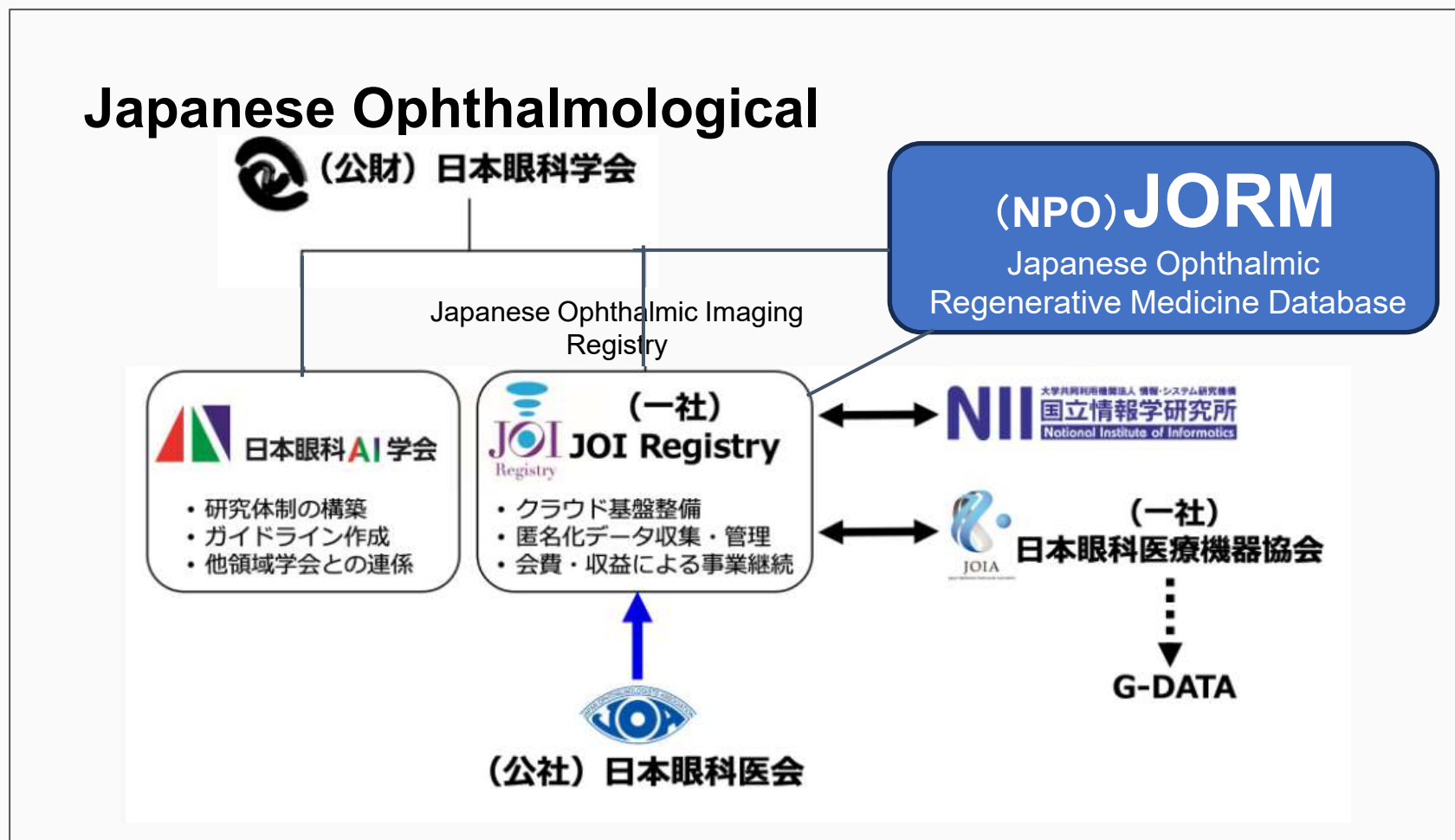
Product ≠ Treatment



Whole treatment Development



Patient Selection Registry for Regenerative Medicine in Ophthalmology



Consortium for RPE transplantation

- Kyoto Univ.
- Kyushu Univ.
- Yokohama City Univ.
- Aichi Med Univ.
- Hiroshima Univ.
- Tokyo Univ.
- Kobe Eye Center
-
-
-

More than 20 Universities

NRMD/CR ⇒ Clinical trial ⇒ PMS data utilization

Japanese Association of
Medical Science



日本医学会
The Japanese Association of Medical Sciences

Registry Council
for Regenerative Medicine

NRMD/CR

NRMD/PMS



case data

As a historical control
Utilized for new development, indication expansion, and (re)review

clinical research

clinical trial

(with conditions
and deadlines)
approval

PMS

Act for Safety of
Regenerative
Medicine,

Pharmaceuticals and
Medical Devices Law



infrastructure
improvement

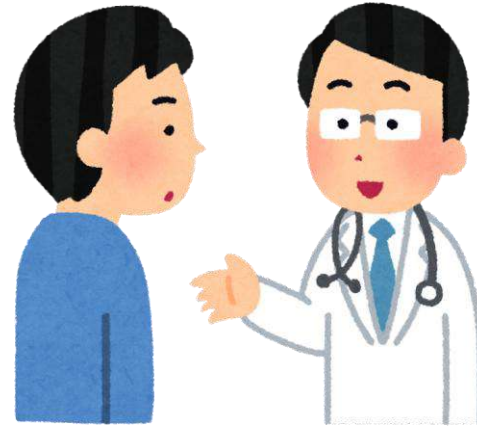


pharmaceutical
consultation

Retinal Regenerative Medicine Explanatory Software

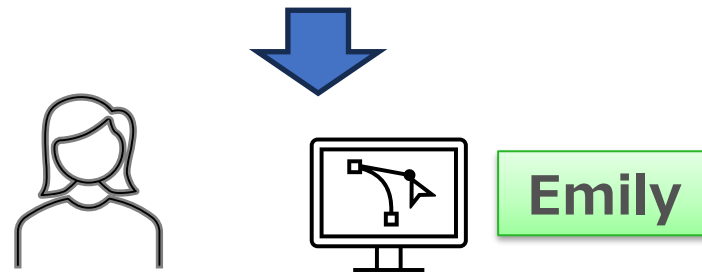
Problem now

- I can't ask questions about what I don't understand.
- Technical words are difficult.



- take time
- It needs to be explained to the patient accordingly.
- Only a physician who knows regenerative medicine well can explain it.

Solution



How much therapeutic effect can be ~

Transplantation of RPE cells made from iPS cells is thought to be ~
~

Whole treatment Development

